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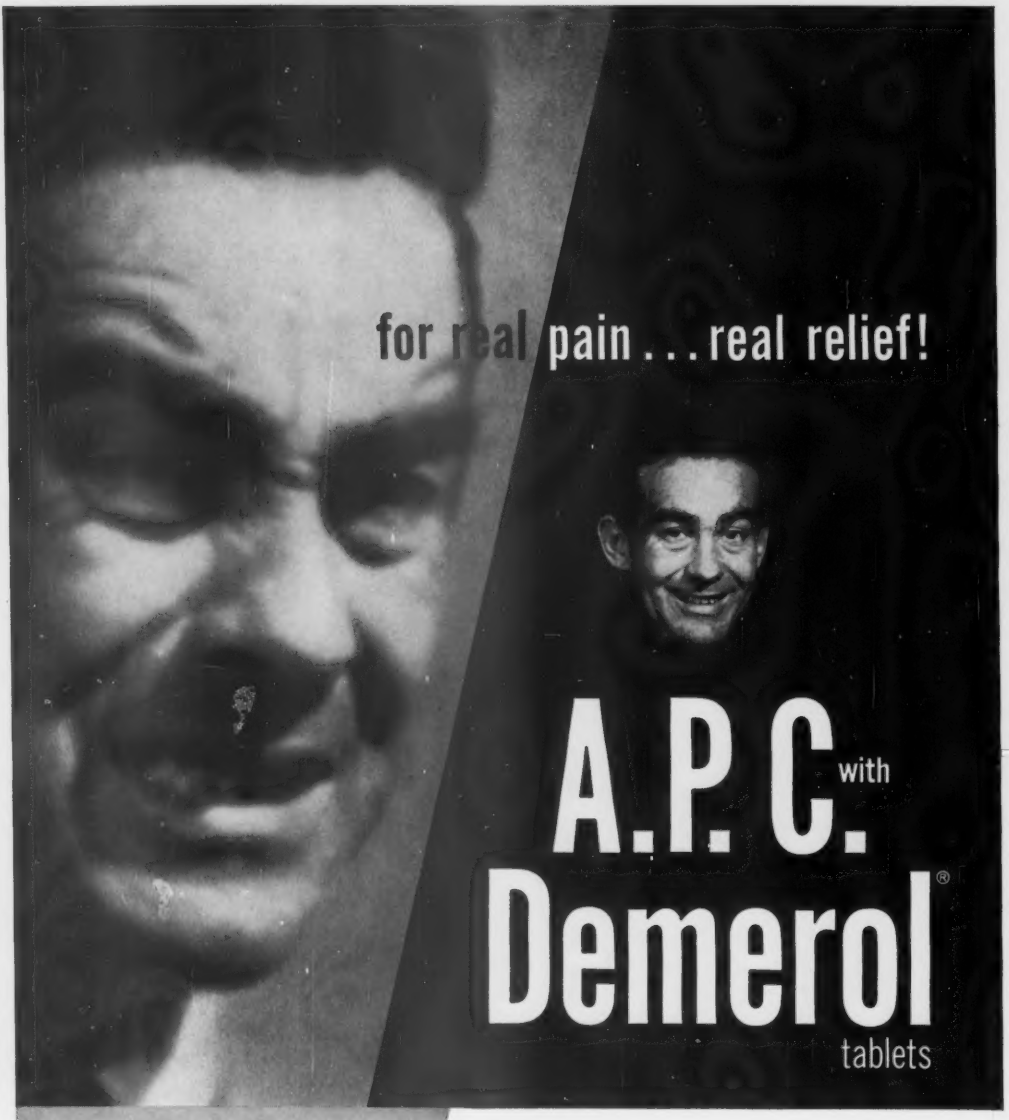

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NICHOLAS J. GIARMAN*

Department of Pharmacology, Yale University School of Medicine

NEUROHUMORS IN THE BRAIN**

About 25 years ago, after Loewi had produced simple, direct and convincing evidence for chemical transmission at neuro-effector junctions in the heart and Dale had provided similar evidence for the neuromuscular junction, Sir Charles Sherrington made it clear that he had foreseen the possibility of chemical transmission at synapses in the central gray matter. At that time (1935) Sherrington told Dale: "I have always assumed that the process at the motor nerve ending must provide a model, or paradigm, for that at the central synapse."[1]

At first it was natural to inquire simply whether acetylcholine (ACh) is *the* central transmitter. Today, in the face of renewed and vigorous investigation in this area, the available evidence will not allow us to assume that ACh is the universal central synaptic transmitter, but it does suggest that ACh is a mediator at numerous central synapses. This is not to say, moreover, that the state of our knowledge is satisfactory and unambiguous. The most decisive evidence for chemical transmission, i.e., the collection and identification of the transmitter after stimulation of the presynaptic neurone, and the demonstration that the presumed transmitter when applied exogenously can elicit characteristic postsynaptic responses, has not been obtained for any specific central synapse. It is worth considering Paton's view that the pharmacological evidence now accepted for central chemical transmission is often weak,[2] but the situation is by no means unpromising.

The design of experiments, the construction of working hypotheses, and general thinking concerning this problem owe a great deal to the wealth of data available on humoral transmission at such peripheral sites as autonomic ganglia, secretory cells, smooth and skeletal muscles. The peripheral findings, however, can only have relevance to events in the central nervous system (CNS) when viewed in the light of data coming directly from the CNS itself; and only recently have such data been forthcoming

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* Associate Professor of Pharmacology.

** This review is not intended to be an exhaustive treatment of the subject, but rather an attempt to incorporate new information into some of the older body of knowledge.

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in any abundance. Nevertheless, there has been a noticeable lack of restraint on the part of investigators lately to ascribe the role of central transmitter to several of the recently discovered natural products of the nervous system without presenting adequate experimental evidence for such a role.

We owe a great deal to Feldberg's clear thinking and careful exposition having dispelled some of the confusion in this area. In keeping with some of his ideas and with those put forward more recently by Elkes,[8] one can establish some minimal criteria which should be met by any naturally occurring substance to be entertained for a role as a chemical transmitter at central synapses.

1. The first of these is that the substance should be present in the CNS, not in a uniform, but rather in a discreet pattern of distribution. If the substance is to be considered a transmitter and not merely a neuroregulatory hormone, it should be ascertained that it is elaborated in the neurone and released from the presynaptic nerve terminal. Furthermore, it is to be expected that local concentrations of the substance will be shown to be related to the function of a given neural structure, and that fluctuation in local concentration will take place in response to functioning of such a structure, or will lead to a quantitative change in the function of the structure.

2. Secondly, there should be in the CNS enzymatic mechanisms for the synthesis of the material, and enzymatic or other mechanisms for its destruction and the dissipation of its action. Such mechanisms necessarily should involve reactions of high velocity, consonant with the rapidity of events in the nervous system. Synthesis need not keep pace with demand, however, if provisions are available for storage of the material in an inactive form.

3. Thirdly, there should be clearly demonstrable effects resulting from a deliberate increase or decrease in the local concentration of the substance. Such a change can be brought about in one of two ways. One can alter the concentration in the brain directly by applying the substance topically to the brain or by administering it into the vascular system. The brain level can also be altered indirectly by the systematic administration of a known metabolic precursor of the substance or of an inhibitor of the destructive enzyme. In all three cases, the administration of the substance itself, its precursor, or the inhibitor of the destructive enzyme, a consideration of the blood-brain barrier enters prominently. It must be ascertained that the appropriate compounds can traverse this barrier.

4. Finally, known blocking agents of the substance should also produce demonstrable effects by virtue of prevention of the transmitter from access to its specific receptor sites.

Largely because of currently productive investigations of chemical substances which alter mood and behavior in man, there has been a resurgence of interest in chemical transmission in the brain. As an outgrowth of this intensive research, has come, in the past few years, an elucidation of the nature of a number of interesting new substances in brain, most of which have been suggested as possible transmitter substances. These include some new esters of choline related to ACh, an amine, 5-hydroxytryptamine

TABLE 1. LEVEL OF ACH IN RAT'S BRAIN DURING VARIOUS STAGES OF CNS ACTIVITY*

Functional state of CNS	*ACh Content (μg./gm.)*			
Deep anesthesia (barbiturate and ether)	1.8	3.9	5.8	5.5
Sleep	1.4	...	...	...
Normal	1.3	2.9	3.5	3.0
Excitement	0.9	...	...	...
Convulsions	0.6	2.2	2.9	...
	Richter and Crossland[65]	Tobias *et al.*[76]	Elliott *et al.*[24]	Wajda[82]

* Adapted from Feldberg.[27]

(5-HT, serotonin), which must be added to epinephrine, nor-epinephrine, and histamine as biogenic amines with potential neurohumoral function, and at least one polypeptide, substance P.

ACETYLCHOLINE

It is perfectly natural to turn one's attention first to the peripheral synaptic transmitter, ACh, about which a tremendous body of information has accumulated. To be sure, ACh is present in the CNS in a non-random distribution; and its concentration does vary with the functional state of the CNS (Table 1).

Apart from the inconsistency in absolute values, which no doubt result from differences in technique of extraction and bioassay, it is possible to see certain trends in the relative values: high levels of ACh seem to obtain during states of CNS depression, while lower levels appear during wakefulness. An important problem which must be met in such studies is whether

there is a causal relationship between the level of the chemical in the brain and the functional state observed. Does the rise in ACh precede and lead to sleep and anesthesia or does the depressed brain cause some imbalance in the dynamic relationship among synthesis of ACh, its storage, its release, and its destruction? Actually, rates of change in concentration of the transmitter would be more revealing than absolute or relative values at any given moment.

There is no longer any doubt about the close relation between electrical activity in the cortex, ACh content, and release of ACh from this area. However, this relationship with respect to frank seizures is apparently a complex one. Richter and Crossland[65] have demonstrated an unusual phasic association of ACh content with convulsive activity. Electrical stimulation produced first a sharp decline in ACh content of the brain followed by a return to almost normal levels before convulsions supervened. Since the time intervals in these experiments were in the order of 10-20 seconds, a massive synthesis, or more probably a massive release, of ACh must be assumed to have taken place.[23]

This brings up a point of importance which applies as well to the CNS as to the peripheral nervous system. Because the transmitter must be a highly reactive chemical and must take part in high velocity reactions, it most probably exists in a pre-formed pool which is held away from the receptor sites (and destructive enzymes) either by protein binding or by anatomic barriers (e.g., intracellular granules). Tower and Elliott[79] have drawn attention to the importance of distinguishing between free and "bound" ACh, and have emphasized the probable role played by ionic forces in regulating the release of ACh from its storage sites. Recently, intracellular granules have been shown to serve as depots for ACh,[48] catecholamines,[8,20] histamine,[62] and 5-HT.[5,37]

It is now definitely established by the work of Nachmansohn and colleagues,[67] of Feldberg and Vogt,[30] and of others that the enzyme which synthesizes ACh, choline acetylase (ChA), and that which hydrolyzes ACh, acetylcholinesterase (ChE), are both present in the CNS. The studies of Feldberg and Vogt[30] are classic and bear some scrutiny. They found a curiously uneven distribution of ACh-synthesizing capacity in the CNS. An interpretation of their findings was made by applying what is known of the properties of cholinergic neurones in the peripheral nervous system. Consideration was given, for example, to the following facts: that a peripheral cholinergic fiber can synthesize ACh along its entire length; that the magnitude of activity of the ACh-forming system in a tract is related to the number of cholinergic fibers contained in the tract; and that a noncholinergic bundle is easily identified by its almost complete lack of

ACh-synthesizing capacity. These workers concluded that in the CNS there is often an alternation between cholinergic and non-cholinergic neurones in both afferent and efferent pathways. This was most convincingly demonstrated[28] in the optic pathway of the dog, where the retina was found to be rich in ChA; the optic nerve, very low; the lateral geniculate bodies, high again; and the visual area of the cortex, intermediate in activity. Further analysis of the retina revealed that the first-order neurone was noncholinergic, while the second-order neurone with its bipolar cell impinging on the ganglionic layers was distinctly cholinergic.

There is substantiation of these findings in the work of Francis[21] and of Anfinson,[3] who made use of a histochemical localization of ChE in the cat's and cow's retina. They found a high concentration of the esterase in the inner synaptic layer of the retina, indicating cholinergic transmission from the bipolar cells to the ganglion cells, or that the second order neurones are cholinergic. The absence of such evidence for ChE in the outer synaptic layer is in agreement with the view of Feldberg and Vogt that the first-order neurone is noncholinergic. It should be emphasized here that findings arising from studies on the destructive enzyme may not be as significant as those obtained from investigations of the synthesizing enzyme because of the rather anomalous finding of ChE in the presynaptic boutons termineaux of ganglia, instead of, as expected, it being in close association with the postsynaptic receptor site (as at the motor end-plate).[48]

An alternating sequence of cholinergic and noncholinergic neurones similar to that in the optic pathway has also been found in the voluntary motor pathway and elsewhere in the CNS.[30] The one criticism which one might make of this work is that it was done at a time when acetyl-coenzyme A had not yet been shown to be the active-acetate donor to the ChA system; so Feldberg and Vogt used ATP to drive their ACh synthesis. Their alternating sequence could conceivably represent an alternation of structures which can or cannot utilize ATP to form acetyl-CoA and ultimately ACh. However, studies of the presence of endogenous ACh in various brain structures bear out the uneven distribution idea; and it has already been pointed out above that investigations of the distribution of ChE substantiate the alternating neurone concept in the optic pathway. Furthermore, a recent work by Hebb and Silver,[41] taking into account that choline acetylase activity is limited by a supply of acetylcoenzyme A, has largely confirmed the results of Feldberg and Vogt.

In addition to fulfilling the first two criteria mentioned earlier, ACh, when applied properly to the CNS, has pronounced central actions.

1. It causes a discharge of motor impulses leading to muscular contractions.

2. It alters the reflex excitability of the CNS.

3. Depending upon the size of the dose, it stimulates or depresses the respiratory center.

4. It has an action on the centers of the autonomic nervous system whereby changes in blood pressure, heart rate, and intestinal movements are produced.

5. Injected into the region of the supra-optic nucleus, it causes a release of antidiuretic hormone.

6. When injected into the ventricular system of the cat, the animal undergoes something similar to an akinetic seizure.

All of these effects are produced by the direct application of ACh to the brain or by injecting it into the vascular system, the latter being a very inefficient way to administer it because the esterases of the blood rapidly hydrolyze it, and because the blood-brain barrier bars it almost completely from access to the CNS. There are, however, two other ways to cause an increase in the local concentration of ACh in the brain.

1. Administration of any one of a large number of potent inhibitors of ChE (such as physostigmine, di-isopropylfluoro phosphate, tetra-ethylpyrophosphate), most of which traverse the blood-brain barrier. Such drugs have been shown to cause up to 200 per cent elevations in brain ACh.[73]

2. At least theoretically the administration of one of the precursors of ACh would lead to an increase in its endogenous level. Choline can be eliminated from consideration of brain ACh, because it cannot penetrate the blood-brain barrier; but a precursor of choline, dimethylaminoethanol, DMAE, is a tertiary amine and should enter the brain from its vascular supply. Investigations in our laboratory, however, have failed to demonstrate the conversion of unlabelled DMAE to brain ACh *in vivo* under a number of conditions, or *in vitro* by rat brain slices, even when potential sources of methyl groups were added to the medium.[66] Nevertheless, DMAE has produced interesting pharmacological effects and behavioral changes in animals,[8] and has led in humans to an elevation of mood and an allaying of fatigue.[68] It is at present under clinical trial as an antidepressive drug in a number of psychiatric disorders.

The central effects observed with the various anticholinesterase drugs, which allow the accumulation of endogenous ACh, represent a series of observations highly pertinent to the question of whether ACh is a central synaptic transmitter or not. There is a large number of such compounds, most of which have been developed for purposes of chemical warfare. One of the more potent and long-lasting of these, di-isopropylfluorophosphate (DFP), has been rather extensively studied for its central actions and

will serve here as a prototype for agents with similar irreversible ChE-inhibiting properties. In man, DFP produces an emotional lability, restlessness, tension, EEG changes and, in large enough doses, convulsions. In general, these effects may be categorized as excitatory; and this is also true in animals, whether intact, anesthetized, decerebrate, or spinal. In large measure, the effects of DFP on the brain and on the spinal cord are similar to those produced by ACh, but, of course, much more prolonged.[25]

Decisive in the argument relating the central effects of anticholinesterases to ChE inhibition in the brain is the fact that although these compounds are chemically dissimilar (physostigmine is a tertiary amine, neostigmine, a quarternary ammonium compound, and DFP, a nonbasic organic phosphate ester), they all produce in low doses the same over-all effect. Since ACh itself produces similar effects and is known to be present in brain, the relationship is strengthened, as is the case for ACh's participation as a central transmitter.

An interesting effect produced in animals by subconvulsive doses of anticholinesterase compounds injected via the carotid artery is an easily recognized and reproducible condition of forced circling movements. These have been antagonized by atropine, and like certain other central changes (e.g., those disclosed by EEG tracings) have been used as a technique for studying centrally acting drugs in the cholinomimetic category. This allows the emergence of one final point in the argument for ACh as a central transmitter. Atropine is generally accepted as the classical antagonist of the muscarinic actions of ACh in the autonomic nervous system. Thus, if ACh is active as a central synaptic transmitter and if atropine can enter the brain, one might expect atropine to exert interesting central effects, related possibly to a central antagonism of ACh. It is known that atropine will prevent the development of spike potentials by topical application of ACh to the cerebral cortex,[27] and that the intraventricular injection of atropine in conjunction with ACh does not result in the spike discharges usually seen when ACh is given alone by this route.[28a]

If given in sufficiently large doses, atropine has definite central actions. There is slowing and increased amplitude in the electrocorticogram[28] reminiscent of the barbiturates. And, like the barbiturates, it prevents the appearance of the arousal reaction; but the situation is paradoxical, because the animal is behaviorally awake and may even appear to be excited. This, nevertheless, has been taken as evidence for a cholinergic mechanism in the reticular activating system.[78]

It is of interest that atropine has been of some clinical value in paralysis agitans and in motion sickness, although certain synthetic compounds,

somewhat analogous structurally to atropine, have been much more effective in these conditions. While the role of ACh in such disorders has not been made clear, the clinical action of atropine may be looked upon as presumptive evidence that ACh does play some role. Of significance in this regard also is the production of toxic psychoses in humans who have had an overdose of atropine. Recently, Abood and co-workers[1] have reported powerful psychotogenic properties of a number of synthetic analogs of atropine.

In recapitulation, it has been pointed out that ACh is present in the CNS; that it is continually released and synthesized; that in minute amounts it exerts a variety of central effects; and, what may be more pertinent, that a number of chemically diverse substances, which have one property in common, the inhibition of ChE, all produce ACh-like central effects; and that a peripheral inhibitor of ACh, atropine, and some of its analogs have powerful central actions. These, it would seem, greatly strengthen the theory that ACh is a central synaptic transmitter, regardless of what interpretation one places upon each single fact.

At this point, the question which has often been raised in the past (but not so often recently) is whether ACh is the only central transmitter or whether there is a whole family of central neuro-humors. Even in cholinergic mechanisms, ACh may be only one of several choline esters of importance. In recent studies, urocanylcholine, acrylylcholine, and dimethylacrylylcholine, and γ-amino-butyrylcholine have all been found to occur naturally and to exert potent effects. The evidence in favor of noncholinergic neurohumoral substances is accumulating rapidly, and is both suggestive and provocative, but far from conclusive. Actually, it is not precise to limit these to noncholinergic systems, because they could conceivably serve as modulators of cholinergic systems as well.

CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE (SEROTONIN; 5-HT)

In terms of the criteria mentioned earlier, two amines of recent study have achieved consideration as central neurohumors of a magnitude almost equal to that of ACh. These are norepinephrine (NE) and 5-HT. They are both present and nonrandomly distributed in the brain (although the presence of 5-HT in a neurone proper has not been demonstrated); there are present in the nervous system rapidly active mechanisms for their synthesis and destruction; and they both produce interesting, diffuse effects when their levels in the brain are increased or decreased. In point of fact there is a new school of thought (Osmond,[58] Woolley[87]) which holds that abnormalities in the metabolism of these amines is the underlying bio-

chemical mechanism in the etiology of certain mental disorders. These concepts rest upon tenuous and indirect evidence, to be sure, but they have stimulated basic research in an area which has been in need of new and imaginative approaches.

It is of interest that the distribution of these two amines in the CNS is quite similar, both being localized in the gray matter of the mesencephalon, diencephalon, and rhombencephalon. One quickly sees this parallelism in Table 2, as well as almost complete lack of correlation with levels of ACh.

TABLE 2. LEVELS IN BRAIN OF DOG AND CAT OF NE, 5-HT, AND ACH RELATIVE TO THE LEVEL IN THE HYPOTHALAMUS, EXPRESSED AS 100%*

Neural structure	*5-HT*	*NE*	*ACh*
Hypothalamus	100%	100%	100%
Amygdala	130	...	...
Septal nuclei	123	...	...
Pineal (Cow-ref. 35)	108	40	33
Area postrema	73	100	...
Hippocampus	70	...	...
Midbrain	61	36	...
Floor 4th ventricle	38	26	...
Superior colliculi	35	16	92
Spinal cord (Gray)	25	16	165
Medial thalamus	20	23	...
Olfactory bulb	20	5	98
Cerebellum	3	6	10
References	62, 61	82	51

* These relative values were calculated from the following levels of the neurohumors in the hypothalamus (μg./g. wet tissue): 5-HT in dog, 0.37[61, 62]; NE in cat, 1.03[62]; and ACh in cat, 1.8.[51]

Another series of findings which tend to link 5-HT and NE have to do with alteration of the brain content of these two amines by certain neuro-active drugs and precursors. For example, the potent tranquilizing drug, reserpine, which is useful in the treatment of psychoses and psychoneuroses (as well as certain forms of hypertension) has been shown to release NE and 5-HT from their storage sites in the brain (and from other body depots).[12, 74, 45] This property of reserpine is shared only by those alkaloids of *Rauwolfia* which also exert a central tranquilizing action.[74] It has been tempting, therefore, to ascribe the central actions of reserpine and its active analogs to the release of NE and 5-HT. In fact, there has

been some recent evidence[18] to show that the soporific action of reserpine in mice can be abolished for a time or prevented by raising the brain levels of HT and NE through the simple expedient of injecting their precursors, 5-hydroxytryptophan and 3, 4-dihydroxyphenylalanine (DOPA), respectively.

This already complex situation is made more complicated by the findings that other centrally acting drugs can influence brain levels of either 5-HT or NE (cf. Table 3).

TABLE 3. INFLUENCE OF SEVERAL DRUGS AND PRECURSORS ON BRAIN CONTENT OF NE AND HT

	Direction of change in brain content of:		
Chemical agent	*HT*	*NE*	*Species-ref.*
Apomorphine	O*	Decrease	Cat[60]
Morphine	O*	Decrease	Cat[60]
Reserpine	Decrease	Decrease	Rabbit[13] Cat[45]
Anticonvulsants	Increase	?	Rat[19]
Barbiturates	Increase	?	Rat[2] Dog[26]
Iproniazid	Increase	Increase (in some species)	Rat[60]
5-Hydroxytryptophan	Increase	?	Rat[9]
"DOPA"	?	Increase	Mouse[18]

* No change in rat's brain content.[60]

Two problems arise from a consideration of these data. The first is whether there is a causal relationship between the change in neurohumoral level observed with a given drug and the pharmacological action produced by that drug. The other relates to such paradoxical situations as a high brain level of 5-HT obtained in an animal *depressed* with a barbiturate or *excited* with iproniazid or 5-hydroxytryptophan. This has been explained by Brodie and Shore[18] on the theory that excess of 5-HT in the brain counteracts its own action. Other possibilities exist, however, in the differential elevation of 5-HT levels in selected areas of the brain or in differential ratios of "bound" to free forms of 5-HT brought about by the drugs in question.[57] The crux of the matter finally may rest not in the changes produced in the level of any single neuro-humor, but rather in the necessary relatedness of these substances, one to the other. It would seem more appropriate at this stage of our developing knowledge to visualize small groups of related compounds which by mutual interaction would control

the phenomena of neural excitation and inhibition, rather than to restrict our thinking to unitary chemical schemes.

It is rather interesting to note that the precursors of NE and 5-HT, dopamine and 5-hydroxytryptophan, respectively, can cause an increase of from 200 per cent to 500 per cent in the brain level of the appropriate amine *in vivo*. The same is true for inhibitors of monoamine oxidase, an enzyme which can destroy NE and 5-HT (cf. the effect of iproniazid in Table 3). However, lethal doses of potent inhibitors of cholinesterase, such as DFP, produce at most, 150 per cent increase in brain ACh, while the precursors of ACh, choline and dimethylaminoethanol, have no detectable influence on brain levels of ACh *in vivo*. It would appear, therefore, that mechanisms controlling brain levels of ACh are more efficient than those controlling levels of 5-HT or NE.

Whether there is a causal relationship between the effects of the drugs in Table 3 and the changes in brain NE and HT or not, it is a well-established fact that exogenously produced changes in the concentrations of these amines in the brain do elicit demonstrable central effects. These may be summarized as follows:

1. NE and epinephrine (E) injected intracisternally, produce analgesia, sleep and anesthesia, and a long-lasting hyperglycemia.[54]

2. Similarly, Sherwood and Feldberg[59] have found that small doses (20-80γ) of NE or E injected into the lateral ventricle of the cat cause a condition resembling light nembutal anesthesia.

3. On the other hand, it has been shown[11] that both NE and E can produce arousal via the reticular activating system.

4. A rapidly induced sedation also follows the intraventricular administration of 5-HT in the cat[54] and in other species.[59]

5. By injecting either the catecholamines or 5-HT into the internal carotid artery of the cat, Marazzi and co-workers have been able to study their action on transmission in the transcallosal pathway.[58] They found that 5-HT, NE, and E are all potent synaptic inhibitors in that order. A similar synaptic inhibition can be achieved by injecting iproniazid ("Marsilid"), a substance which spares 5-HT from destruction by monoamine oxidase. These workers interpret their results as indicating that 5-HT is a central synaptic inhibitory transmitter.

It is of interest in regard to the enzyme monoamine oxidase, that iproniazid and other potent inhibitors of this enzyme recently have been found to exert a stimulatory action on the CNS and to ameliorate the general mental state and behavior of chronic apathetic schizophrenia and other psychotic depressions.[52] These drugs have been called psychic energizers or antidepressives because of this action. It has been tempting to ascribe

the mechanism of such drugs to their monoamine oxidase-inhibiting activity and the subsequent accumulation of 5-HT and NE in the CNS. But there may be other related amines, not identified as yet in the CNS, which could be oxidatively deaminated as well as 5-HT and NE by monoamine oxidase, and therefore also could accumulate as a result of inhibition of the enzyme. Udenfriend, e.g., has recently demonstrated the presence of tryptamine and other amines in the urine of mammals after the administration of iproniazid.[81] In addition, iproniazid and other monoamine oxidase inhibitors may have other pharmacological actions. For example, it has been shown that iproniazid can inhibit, to some extent, the reserpine-induced release of 5-HT from brain cells.[37]

Finally, one might add that certain substances related structurally to 5-HT and certain substances antagonistic to the actions of 5-HT or NE produce interesting central effects. Noteworthy among these are the following:

1. Bufotenine, the dimethyl analog of 5-HT, harmine, ibogaine, and psilocybin are all naturally occurring indolic alkaloids which produce hallucinations and behavioral changes in man. Their chemical relationship to 5-HT may be seen in Figure 1.

2. Lysergic acid diethylamide (LSD-25), another indole derivative (Fig. 1), is a powerful 5-HT antagonist in some peripheral systems (e.g., on the uterus) in one dosage range and mimics the action of 5-HT in another. Minute oral doses of LSD-25 (75-100γ) characteristically produce bizarre mental aberrations, resembling schizophrenia, in humans.

3. Morphine and other narcotic analgesics (dihydromorphinone, methadone, pethidine, codeine, and ethylmorphine) have been shown to exert a mild antagonistic action on the 5-HT-induced contraction of the guinea-pig's ileum.[56] This effect parallels the analgesic potency of the drugs tested. In this regard, it is interesting that reserpine, which depletes the brain of 5-HT and NE, can inhibit the analgesic action of morphine, while iproniazid, which causes the accumulation of the two amines, can potentiate morphine analgesia.[73]

4. Dibenamine and dibenzyline, both NE antagonists in the periphery, have been used as tranquilizing drugs with mild success and are serving as models for the development of new tranquilizers.[83]

HISTAMINE

Other, less well-explored substances in the CNS are now under some scrutiny. Histamine and substance P, a polypeptide, have been found randomly distributed in the brain and present in the posterior roots. The

possibility that histamine may be a transmitter of the polysynaptic reflex arc has already been suggested.[86] The interesting point about histamine is that it seems to increase in amount as one goes peripherally from the brain (0.1 to 1 γ/gm.) to the spinal cord (1 to 2 γ/gm.) to the spinal roots (4 to 11 γ/gm.) out to certain postganglionic sympathetic nerves

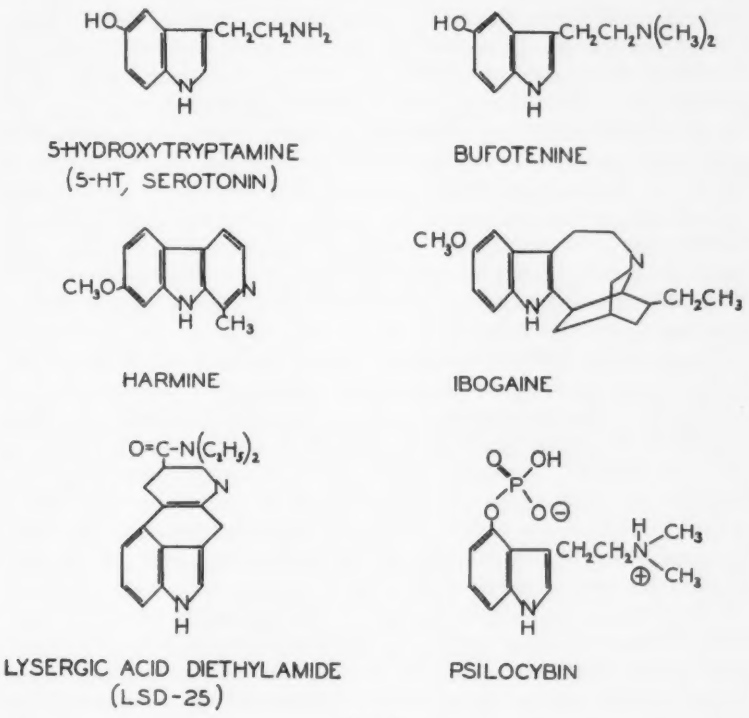

FIG. 1.

(as high as 100 γ/gm. in the splenic nerves of the ox[85]). This distribution may be related to the distribution of mast cells, which are few in the CNS but plentiful in the perineurium and endoneurium of peripheral nerves.[86] However, studies on desheathed nerves and on nerves with few mast cells and high histamine content (like the vagus) indicate that not all neural histamine is contained in mast cells.

Some areas of the brain, moreover, are relatively rich in histamine: optic nerves,[85] hypothalamus,[89] and the pineal gland.[85] In addition, histamine

does have some central excitatory action, and small doses cause a pronounced increase in the electrical activity of the cerebellum.[71] It has also been shown that histamine will stimulate the superior cervical ganglion and the central cell stations of the sympathetic system.[80]

One of the major problems to be reckoned with in assigning a neurohumoral role to histamine is concerned with the mechanisms for the bioformation and biotransformation of the amine in nervous tissue. These mechanisms are poorly explored in the organism as a whole although Werle has shown that some nerves (vagus, pre- and postganglionic sympathetic fibers) can make histamine by decarboxylation of histidine.[84] The content of histamine in various neural structures of the cerebrospinal axis, and the histidine decarboxylase and histaminase activity of these areas need to be systematically investigated before further emphasis can be placed on histamine as a neurohumor. The fact that histamine is distributed uniformly along peripheral nerve fibers and not concentrated in their terminals makes it unlikely that histamine is liberated as a mediator of nerve impulses. West views histamine rather as a line of defense against injury possessed by certain nerves.[90]

The possibility exists that histamine is a precursor of another active material which may play some role in nerve function. It has been observed that histamine-like activity in extracts of the sympathetic trunk from the ox may be inactivated by treatment with alkali, which ordinarily does not destroy histamine.[85] The recent finding that histamine is N-methylated by imidazole N-methyl transferase of mouse liver is also of some significance in this regard,[14] because earlier it had been shown that N-methyl histamine is a good substrate of monoamine oxidase and can be spared from breakdown to 1-methyl-imidazole-4-acetic acid by iproniazid.[72]

It is now well established that various antagonists (in peripheral systems) of histamine have interesting and useful central actions; but the relationship between effects on the nervous system and a central antagonism of histamine is entirely presumptive. A number of these drugs have been used effectively as anti-emetic agents, as hypnotics and sedatives, and, more recently, as tranquilizers (e.g., hydroxyzine). In evaluating such actions in terms of neurohumoral antagonism, it is well to remember that the so-called antihistamine drugs are often mild antagonists of ACh or NE or both.

SUBSTANCE P

The polypeptide, substance P, occurs in appreciable concentration in the brain with highest values in the area postrema, hypothalamus, basal ganglia, and gray matter of the medulla spinalis.[64] It has also been found in

the retina of the cow and dog. In the dorsal roots, Hellauer and Umrath have found a vasodilator factor to which they have ascribed a transmitter function.[48] Whether this is the same as substance P which also occurs in this site, has not yet been ascertained. Nevertheless, Kopera and Lazarini consider substance P to act as a chemical mediator of nerve impulses in the CNS.[48]

Little is known about the mechanisms of bioformation and destruction of substance P either in the CNS or elsewhere, although presumably the usual pathways for peptide synthesis and degradation are involved. However, it is known that administration of the material does evoke certain central effects. Intracisternal or intraventricular injections of small doses of a highly purified preparation elicit stimulation of respiratory frequency and amplitude in cats and rabbits. Injected into the lateral ventricle, substance P produces a variety of signs marked by a general inhibition of spontaneous activity.[26] Moreover, changes in the EEG of the cortex (decreased amplitude, increased frequency) and of the hippocampus of the rabbit by the intracarotid administration of small amounts of substance P have been interpreted to indicate an influence on activating systems.[80] It is rather intriguing that the distribution of substance P is similar to that of 5-HT and NE except for its concentration in dorsal roots. In view of their concentration in area postema, hypothalamus, and medial thalamus, all three substances are considered to have an "equally good (or bad) claim to reticular function."[26]

GAMMA-AMINOBUTYRIC ACID

One of the most intriguing of recent developments in neurochemistry has been the elucidation of the presence in brain of a relatively simple amino acid, γ-aminobutyric acid (GABA), and the demonstration of its inhibitory synaptic activity. A few years ago Florey had reported upon the inhibitory activity of extracts of brain and spinal cord on the generation of impulses by the stretch receptor neurone of the crayfish.[28] The agent (or agents) responsible for this action was called Factor I; and only recently a major component of Factor I was identified as GABA.[7] It is remarkable that Hayashi and Nagai, studying a number of related compounds for anticonvulsant activity, chose γ-amino-β-hydroxybutyric acid as a potential central inhibitory substance.

The presence of GABA in brain has been known since 1950; until recently it has been considered mainly from the point of view of its role in intermediary metabolism. It was shown that GABA is produced in brain from glutamic acid under the catalysis of a glutamic acid decarboxylase, which requires pyridoxal phosphate as a co-factor.[70] The dissipation of

GABA depends mainly upon its transamination with α-ketogluturate to produce glutamic acid and succinic semialdehyde.[69] This latter substance may enter the tricarboxylic acid cycle and thus be utilized for energy production. However, GABA itself has been shown recently to support oxygen utilization by slices of the cat's cerebral cortex.[105]

Uniquely high quantities of GABA (approximately 400 γ/gm.) have been found in an easily extractable form in the CNS of representative species of vertebrates but not in other tissues. The possible function and importance of this substance in the CNS, however, is still a matter of speculation. The depressant action of GABA when applied to the cortex of normal animals and the anticonvulsant activity of GABA in mice has focused attention on a possible role which it may play in seizure and antiseizure systems in the brain. Convulsant hydrazides, such as thiosemicarbazide, which inactivate pyridoxal, have been shown to produce a decrease in the activity of glutamic acid decarboxylase and in the content of GABA in the brains of rats and to decrease the rate of formation of GABA from glutamic acid *in vivo* in mice.[47, 71] Yet it has recently been reported that there is no correlation between seizure-susceptibility of certain areas of the brain and their content of GABA.[8] It may be premature to implicate only GABA in seizures produced by thiosemicarbazide and other pyridoxal-inactivating compounds, because it is well-established that pyridoxal is a co-factor for the decarboxylases which form 5-HT and dopamine (leading to the formation of NE). It is possible, therefore, that these amines are equally important in the problem of seizures. Furthermore, semicarbazides have been shown also to inhibit histaminase.[4]

Other workers have shown that topically applied GABA reverses surface negative postsynaptic potentials of the apical dendrites evoked by various stimuli in the cerebral cortex of unanesthetized cats.[46, 67] This effect is probably restricted to the surface layers of the cortex.[46] Since it had been proposed that electrocortical arousal is associated with the generation of inhibitory postsynaptic potentials in the apical dendrites of the cortex, Magoun and his colleagues looked for a role for GABA in such arousal but concluded that it had none.[21]

Thus, it is still problematic whether GABA is concerned with the regulation of physiological activity of the brain and other nervous structures. The very high levels of this amino acid in the central gray matter might represent a store of a precursor of an unknown, active metabolite. In this regard, the finding in dog's brain of a substance identified as γ-aminobutyrylcholine[49] would appear to be highly significant. The proof of identity of this ester was far from rigorous, however; and the relatively long sojourn of the compound in the organism due to an absence of esteratic cleavage,

as shown in human blood, in cat's blood, and in mouse brain,[11] would detract from the possibility of its playing an important role in neurophysiology.

Events in the central synapses are characterized by rapid firing rates (up to 1,000 per second have been described) and relative transience in course. In this light, none of the substances mentioned above, with the exception of ACh, is acceptable as a synaptic transmitter, because of their relatively prolonged action. It might be argued, however, that transience of action is not as essential to the functioning of an inhibitory transmitter as it is to a facilitatory one. In any event, one must attach some importance to the fact that these substances do exist in the CNS in interesting distributional patterns.

It is well accepted that the CNS is incredibly complex functionally and anatomically. Therefore, it does not seem reasonable to expect it to be so simple chemically as to be provided with a single universal transmitter. It is most likely that there exists a whole family of transmitters and a number of neuroregulators which may modulate the reactivity of neurones to these transmitters.

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SKIN OXYGEN TENSION DURING ADMINISTRATION OF EPINEPHRINE AND NOREPINEPHRINE TO NORMAL MAN‡

Epinephrine and norepinephrine are biologically active substances which are normally reflexly released in response to a number of stressful situations including hypoxia,[7, 11, 15] hypercarbia,[17, 19] hemorrhage, [6, 7, 12] and general anesthesia.[15, 18, 20] In the process of evaluating the effects of such stressful situations on the amount of oxygen in tissue and available for normal cellular respiration and metabolism, it became necessary to differentiate between those changes in tissue oxygenation which were the result of the stressful situations per se, and those changes in tissue oxygenation which were the result of the reflex release of epinephrine and norepinephrine occasioned by the stressful situations. The present study was instituted to clarify this problem by measuring tissue oxygen concentration (in terms of tissue oxygen tension) in normal adults given infusions of epinephrine and norepinephrine at a time when they were breathing room air at rest. Previous reports on this subject have dealt only with dogs and have been preliminary in nature.[6] Polarographic techniques were used. Although they have certain definite and inherent limitations,[8, 13] when these limitations are respected, such techniques provide valuable information presently unobtainable in any other manner. The present data relate only to changes in skin oxygen tension.

METHODS

Polarography. The method employed for measuring tissue oxygen tension is related to the fact that when a platinum electrode is inserted into a liquid or a tissue and is then incorporated into a circuit, application of a fixed low voltage results in reduction of oxygen about the electrode. The resulting current is proportional to the amount of oxygen present. In the present study open-tipped platinum electrodes were made and

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incorporated into a circuit which had been modified as previously described[4] from the method of Montgomery and Horwitz.[18] After the electrode had been inserted into the skin, a nearby indifferent electrode was applied and a known voltage introduced to complete the circuit. The resulting current was recorded on a Rubicon spot galvanometer. The voltage applied was graduated from 0.2 volt to 0.8 volt in increments of 0.2 volt in order to assure that the resulting amperage fell within the plateau portion of the established oxygen reduction curve.[2] The mid-portions of our plateaus were 0.6 volt as reported by other investigators.[14] Galvanometric readings were taken 20 seconds after the voltage had been applied to allow establishment of equilibrium within the circuit. Readings were taken at intervals of 2 minutes to allow recovery of the electrode. Responsiveness of electrodes as well as electrode decay were checked as previously described.[4] Indifferent electrodes of saline-silver-silver chloride were used throughout. Results are quoted in terms of galvanometric units, the sensitivity of the galvanometer being constant throughout each experiment. Because of the well-recognized difficulties in calibrating such electrodes in tissue,[10] no attempt has been made to interpret amperage (as expressed by galvanometric units) in terms of oxygen tension (as expressed in mm.Hg). Nevertheless, determinations of both magnitude and direction of changes in galvanometric readings indicate proportionate changes in tissue oxygen tension and so, although quantitative results are not possible, reliable qualitative data are obtained.

Clinical. Six normal healthy adult volunteers aged 29 to 37 without clinical evidence of cardiovascular or endocrine disease were studied at rest in the supine position. At the start of each experiment a slow intravenous infusion of 5 per cent dextrose in water was started in an upper extremity and the platinum electrode was inserted into the skin of the lateral aspect of the calf. The electrode was placed in the skin through a small intracutaneous puncture made with a 25-gauge needle. Care was taken to avoid bleeding, subcutaneous insertion, "tenting" or other distortion of the skin, and motion of the electrode. If any of these occurred, the subject was eliminated from further study. Following a period of 30-45 minutes to allow basal conditions to be achieved, base-line readings of skin oxygen tension were recorded and continued at two-minute intervals until constant values had been obtained on three or more consecutive measurements. As soon as a base-line had been established, an infusion of norepinephrine bitartrate (Levophed [CR]) was started through a three-way stopcock attached to the intravenous needle. The rate of infusion was regulated so as to result in an approximately equipressor response in all subjects as determined by sphygmomanometry. In five of the subjects the elevation of systolic blood pressure was 30-35 mm.Hg. In the sixth, the systolic pressure rose 60 mm.Hg. The dosage of norepinephrine, expressed as free base, varied from 0.10 to 0.30 μg./Kg./min. As soon as oxygen tension levels had become stabilized for 5-15 minutes, the norepinephrine was discontinued and the slow infusion of dextrose in water reinstituted. During the recovery period, following norepinephrine infusion, galvanometric readings were taken every two minutes. One-half hour to 45 minutes after the readings had returned to control levels, an intravenous infusion of epinephrine was started and the effects on skin tension recorded. Again, equipressor doses were administered. All subjects showed rises in blood pressure of 25-50 mm.Hg systolic. Dosages used varied from 0.11 to 0.25 μg./Kg./min. When repeated observations indicated that stable oxygen tensions had been achieved following epinephrine infusion, the infusion was maintained for 5-15 minutes and then discontinued. Galvanometric readings were continued during the post-epinephrine recovery period until they returned to control levels. The epinephrine used was U.S.P. epinephrine which has

the disadvantage that it may contain traces of norepinephrine. Nevertheless, the cardiovascular response, especially that of the diastolic pressure and pulse rate following the infusion of the epinephrine, was so characteristic of epinephrine that the amount of norepinephrine included, if any, was physiologically unimportant.[8]

RESULTS

Norepinephrine. All six subjects showed the same response to infusions of norepinephrine. The systolic blood presssure rose an average of

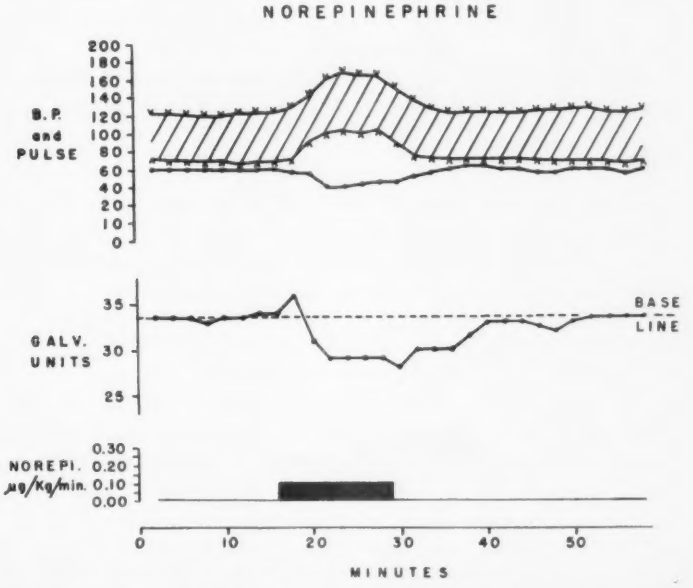

FIG. 1. Effect of norepinephrine infusion on skin oxygen tension in a normal subject. Ordinate (from above down): blood pressure in mm.Hg (V systolic; Λ diastolic) and pulse rate per minute; skin tension in galvanometric units; rate of norepinephrine infusion (µg. of free base/Kg./min.). Abscissa: time in minutes.

37 mm.Hg, the diastolic rose an average of 31 mm.Hg, and the pulse rate decreased an average of 18 beats per minute. Skin oxygen tensions showed a slight and very transient increase in three patients at the start of infusion, followed within 2-4 minutes by a decrease below control levels. When this initial rise did occur, it was of the order of magnitude of 2 to 10 per cent. In the other three patients, there was no initial rise in skin oxygen tension but, instead, the oxygen tension decreased immediately. In all

subjects skin oxygen tension was decreased during the period of elevated blood pressure. The decreases averaged 26 per cent, with individual subjects showing decreases ranging from 10 to 51 per cent. A characteristic response is shown in Figure 1. (It should be borne in mind that the important data in such a study are the percentile changes in skin oxygen tension from control levels, rather than changes in terms of number of galvano-

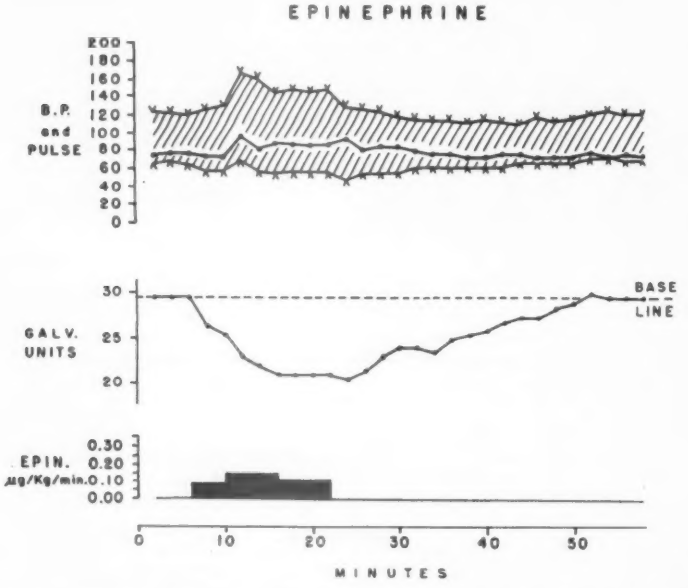

FIG. 2. Effect of epinephrine infusion on skin oxygen tension in a normal subject. See description accompanying Figure 1.

metric units, because base-line recordings vary from individual to individual depending upon the sensitivity of the galvanometer.) Following cessation of norepinephrine infusion, the blood pressure returned to pre-infusion levels within 4-18 minutes but skin oxygen tension required 20-30 minutes to return to control levels.

Epinephrine. The response to epinephrine infusion was also the same in all six subjects. The systolic blood pressure rose an average of 34 mm.Hg, the diastolic pressure decreased an average of 6 mm.Hg, and the pulse increased an average of 14 beats per minute. In two instances there were small, transient increases in skin oxygen tension at the start of the

epinephrine infusion amounting to 4 and 5 per cent. In the remaining four subjects, skin oxygen tension never rose even momentarily but promptly decreased. All six patients showed lowered skin oxygen tensions during epinephrine infusion. The average decrease was 28 per cent, the range —5 to —56 per cent. A typical response is shown in Figure 2. In one patient the appearance of premature ventricular contractions terminated the experiment, but no arrhythmias were noted in other subjects. As with norepinephrine, skin oxygen tensions did not return to normal for 20-30 minutes after cessation of the infusion, although the blood pressure returned to control levels within 4-8 minutes.

DISCUSSION

Tissue oxygen tension is a function of: (i) the rate at which arterial blood is delivered to tissue; (ii) the amount of oxygen in arterial blood; (iii) the rate at which oxygen is consumed by tissue; and, (iv) the coefficient of oxygen diffusion in tissue fluids. Little is known regarding the fourth factor, but the other three are known to be related to a large number of physiological variables. An incomplete list of these variables would include: concentration of oxygen in the inspired air, oxygen-carrying capacity of blood, cardiac output, blood pressure, the state of the peripheral vasculature (whether vasodilated or vasoconstricted), and the presence or absence of calorigenic substances such as thyroxine or epinephrine.

The present results show that skin oxygen tension decreased during infusions of epinephrine and norepinephrine which were adequate to produce a vascular response. The etiology of these decreases cannot be determined at present inasmuch as the experiment was not designed to measure all the variables which can influence skin oxygen tension. Nevertheless, the best explanation for the decreases seen with norepinephrine is that the drug acts primarily by decreasing the rate of delivery of arterial blood to skin rather than by effecting alveolar oxygen tension, arterial oxygen tension, or rate of cutaneous oxygen utilization. The decrease in cutaneous blood flow could be a result of intense vasoconstriction, decreased cardiac output,[1] or both.

Epinephrine has such a marked calorigenic effect that the observed decreases in skin oxygen tension might be explained solely on the basis of increased oxygen utilization. Whether the vascular effects of epinephrine contributed to the decreases in oxygen tension is impossible to determine in the absence of simultaneous measurements of cutaneous blood flow. As Folkow[5] and others have emphasized, the vascular response to epinephrine varies in different organs and, in the case of skin, even in different areas

of the same organ. In addition, the response to epinephrine differs according to species, and also according to route of administration and dosage. It is, therefore, not known whether, in the subjects studied, cutaneous vasodilation or vasoconstriction was present. If the latter, decreases in blood flow and hence in oxygen tension might be anticipated, but vasodilation has also been shown to be associated with decreases in cutaneous oxygen tension under certain circumstances.[4]

Since the catechol amines studied have been shown to be associated with decreases in skin oxygen tension averaging approximately 25 per cent, it is now possible in future studies to apply this fact and so to recognize the extent to which changes in skin oxygen tension are related to reflex release of epinephrine and norepinephrine.

In conclusion, it should be emphasized that the results presented cannot be applied to oxygen tensions in physiologically more important tissues such as the myocardium, the central nervous system, the kidney, and the liver. Such data are the subject of future investigations.

SUMMARY

Epinephrine and norepinephrine infusions in normal volunteers were associated with average decreases in skin oxygen tension of 28 and 26 per cent, respectively, at a time when a vasopressor response had been obtained.

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EFFECT OF ACUTE POTASSIUM DEFICIENCY ON SUSCEPTIBILITY TO INFECTION WITH PARTICULAR REFERENCE TO THE KIDNEY‡

Recent clinical reports describe the association of pyelonephritis and potassium deficiency, and the suggestion has been made that potassium depletion enhances the susceptibility of the kidney to infection.[11, 13]

There are both structural and metabolic alterations in potassium deficiency favoring renal infection. It has been well demonstrated in animals that potassium deficiency produces obstruction of collecting tubules in the medulla secondary to epithelial proliferation and interstitial fibrosis.[13] This results, in effect, in an internal hydronephrosis with stasis of tubular fluid. In addition, potassium is essential in many cellular processes. Lack of this ion could adversely affect the inflammatory process through impaired function of phagocytic cells or cellular mechanisms responsible for the elaboration of humoral factors. Furthermore, it has been shown that increased production of ketone bodies stimulates bacterial growth in tissues.[4, 5] The development of ketosis might be enhanced in potassium deficiency owing to inhibition of carbohydrate metabolism[7] and extracellular alkalosis.[10]

Evidence presented in this report suggests that in the presence of enterococcus, acute potassium deficiency appears to increase the susceptibility of rats to infection in general, with no primary effect upon the kidney.

MATERIALS AND METHODS

White male Sprague-Dawley strain rats weighing 100 to 250 gm. and white male Swiss strain mice weighing about 30 gm. were used .

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The potassium-deficient diet contained approximately 79 per cent carbohydrate, 15 per cent protein, and 5 per cent fat with vitamin supplementation and a basic salt mixture deficient in sodium, potassium, and chloride. The control diet for pair-fed animals had balanced quantities of potassium salts added. Animals on these diets received normal saline *ad libitum*. All other animals were fed Purina Lab Chow.

The strain of *Escherichia coli* employed was originally isolated from the urine of a patient with pyelonephritis. The method of preparing and maintaining this organism has been described.[9] Bacteria for intravenous injection were incubated in beef heart infusion broth at 37° C. for four hours. The volume injected was 0.5 cc. in rats and 0.25 cc. in mice.

The strain of enterococcus (*Streptococcus faecalis*, Lancefield Group D) was obtained from a patient with a urinary tract infection. Bacteria for injection were incubated in beef heart infusion broth at 37° C. for 24 hours. The volume injected was 0.5 cc. of a 1 : 10 dilution.

To enumerate each inoculum, tenfold dilutions in saline were incubated in agar pour plates. The tail vein was used for all intravenous injections. The organisms were identified by colony characteristics, Gram-stain, pigment production, and the use of special media such as Simmon's citrate and Kligler's iron agar.

Organs for bacterial counts were removed under pentobarbital anaesthesia. The abdomen or thorax was opened and the organs removed and placed in a Ten Broeck grinder. The whole left kidney was employed, whereas with lung or spleen about 1 gm. of tissue was removed. The tissue was ground in 9 ml. of saline until a smooth suspension was obtained. Subsequent tenfold dilutions were made in saline, and agar pour plates were prepared from a few of these dilutions, depending on the expected number of bacteria. Colony counts were taken 48 hours after incubation.

The right kidney in all animals and portions of lung, liver, spleen, pancreas, and bowel from some animals were fixed in 10 per cent formalin. All histological sections were stained with hematoxylin and eosin, and the Brown-Brenn method for identification of bacteria in tissues was used when indicated. In animals with high bacterial counts, two or more sections of kidney were examined histologically. Evidence for infection was the association of high bacterial counts with histological findings of acute inflammation, i.e., the exudation of plasma and polymorphonuclear neutrophils and tissue destruction.

Blood was obtained by direct puncture of the abdominal aorta, and serum potassium was determined with a Baird internal standard flame photometer.

RESULTS

In the first group of experiments (Table 1), potassium-depleted rats were injected with a strain of *E. coli* which does not produce pyelonephritis in the normal kidney but causes infection in the obstructed kidney.[9] These animals weighed approximately 250 grams and were maintained on a potassium-deficient diet for two to eight weeks. Serum potassium was significantly low in all groups and the kidneys showed prominent structural alterations attributed to potassium depletion.[13] The intravenous inoculum contained 150 to 200 million organisms, and colony counts were made after intervals of three days to two weeks. Most kidneys contained no more

TABLE 1. COLONY COUNTS IN POTASSIUM-DEPLETED RAT KIDNEYS FOLLOWING INTRAVENOUS INJECTION OF E. COLI

Group	*Length of deficiency*	*Serum potassium mEq/L.*	*Time interval*	*Colony counts x 10^5*
1	4 weeks	2.0-2.6	3 days	400
				151
				111
				11
				10
				1
2	2 weeks	2.3-2.6	1 week	.19
				.10
				.02
				.01
				.01
				0
3	3 weeks	2.4-2.6	1 week	.30
				.20
				.01
				0
				0
4	4 weeks	1.7-2.6	1 week	168
				53
				38
				16
				15
				7
5	8 weeks	2.3-2.5	1 week	15
				1
				1
				.16
				0
6	4 weeks	2.1-2.4	2 weeks	.68
				.03
				.02
				0
				0
				0

organisms than were found in normal animals.[8] A few counts, those over 100,000 in Groups 1 and 4, were higher than those encountered normally;[9] however, evidence of infection was not seen histologically. Furthermore,

TABLE 2. COLONY COUNTS IN NORMAL AND POTASSIUM-DEPLETED MOUSE KIDNEYS FOLLOWING INTRAVENOUS INJECTION OF *E. Coli*

Time interval	*Normal* $x\ 10^3$	*Potassium depleted* $x\ 10^3$	*Serum potassium (K-depleted) mEq/L.*
0	1000		
	800		
	9		
3 hours	43		
	34		
	2		
8 hours	4	11	1.8
	3	9	
		.60	
24 hours	60	74	
	.27	9	2.3
		2	
4 days	.26	24	
	.14	9	2.1
	.10	.24	
1 week	1	3.70	1.7-1.8
	0	1.76	
	0	.78	
		.15	
		.13	
		.05	
		0	
		0	
		0	
		0	

these counts were much lower than those of obstructed kidneys with frank infections.[8]

Because of the negative results obtained after the injection of *E. coli* in potassium-deficient rats, it was decided to study the effect in potassium-depleted mice. Control mice and mice maintained on a potassium-free diet for four weeks were injected with 75 to 100 million organisms. The results are represented in Table 2. Up to one million organisms were recovered

in normal animals immediately post-injection, which represent chiefly bacteria in the blood circulating through the kidneys at this period. Over the next few hours and days the number diminished, and at one week viable bacteria were recovered in only one of three animals. Bacterial counts were not significantly different in potassium-depleted mice, and evidence of infection was not seen histologically. The kidneys showed prominent alterations secondary to potassium lack.

Data presented thus far indicate that both potassium-depleted rats and mice had no increased susceptibility to renal infection after the intravenous administration of *E. coli.* Amren,[1] while studying experimentally induced

TABLE 3. OCCURRENCE OF RENAL AND OTHER INFECTIONS IN CONTROL AND POTASSIUM-DEPLETED RATS INJECTED WITH ENTEROCOCCUS

	Control			*Potassium depleted*			*Serum*
Group	*No. of animals*	*Renal infection*	*Other* infection*	*No. of animals*	*Renal infection*	*Other* infection*	*potassium mEq/L.*
1	10	2	..	10	4	..	2.0-2.4
2	10	2	3	10	4	6	1.8-2.3
3	10	2	5	10	5	8	2.1-2.3
4	10	1	3	10	7	9	1.9-2.4
Total‡		5	11		16†	23†	

* Includes pleuritis, mediastinitis, peritonitis, pancreatitis, and septic spleen.
† Significantly different from control group ($p < 0.05$) by chi-square test.
‡ Total includes only Groups 2 to 4.

bacterial endocarditis, found that the strain of enterococcus employed was capable of producing renal infection in normal rats. It was of interest to determine whether this organism would produce a higher incidence of pyelonephritis in potassium-deficient animals. An inoculum containing 30 to 50 million organisms was used since this caused renal infection in about 20 per cent of normal rats. Animals used in this study weighed about 100 gm. and since they were in a phase of rapid growth, two weeks' maintenance on a potassium-deficient diet produced significant lowering of serum potassium (Table 3) and marked structural change in the kidney. Bacterial counts were done four to six days' post-injection, although in several instances they were done earlier when death occurred or when death appeared imminent.

During the course of the first experiment (Group 1, Table 3) it became evident that infections were occurring in sites other than the kidney. Complete bacteriological and histological data were not obtained in this group, so that only the incidence of pyelonephritis was ascertained. Infection was

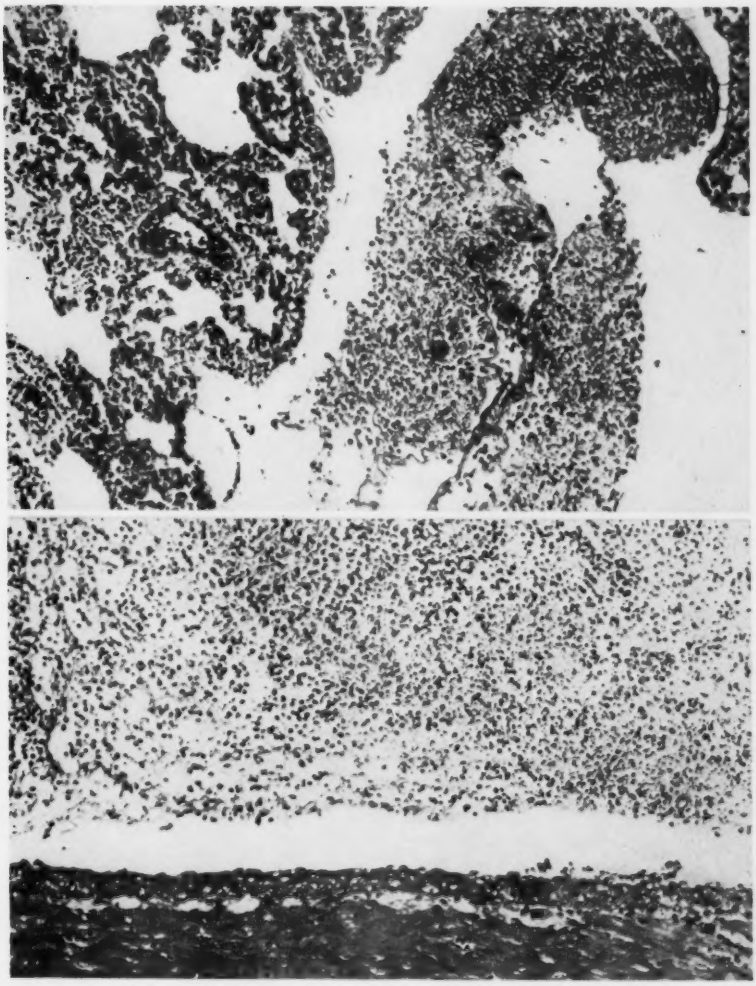

FIG. 1. Types of infections in potassium-depleted rats after the intravenous injection of enterococcus.

A. Mediastinitis. Heavy polymorphonuclear infiltrate in mediastinal tissues. H. and E. x100.

B. Peritonitis. Thick fibrinopurulent exudate adjacent to serosa. Bowel wall below. H. and E. x100.

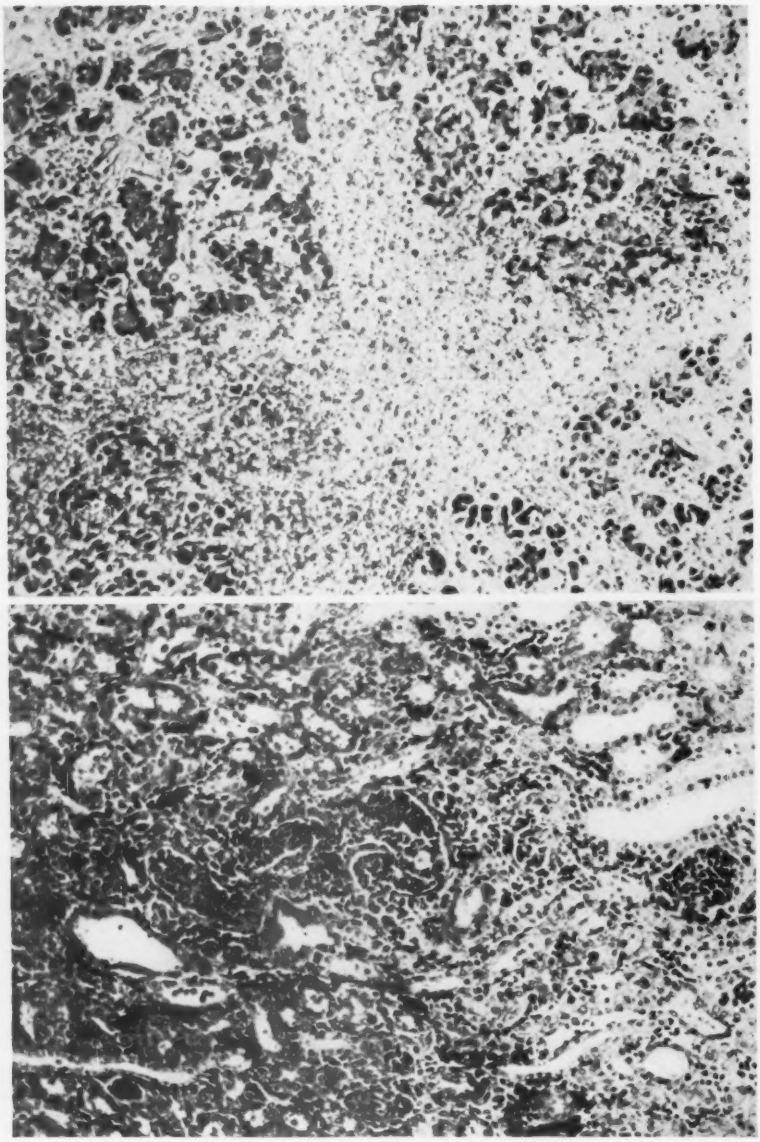

FIG. 1 (continued). Types of infections in potassium-depleted rats after the intravenous injection of enterococcus.

C. Pancreatitis. Extensive interstitial exudation and focal acinar necrosis. H. and E. x100.

D. Small focus in mid-medulla with slight acute inflammatory exudation in the interstitium and several necrotic tubules filled with masses of polymorphonuclear cells. H. and E. x100.

localized in the peritoneum, pancreas, retroperitoneum, spleen, pleura, and mediastinum. With such multiple involvement, evidence of infection was recorded as positive only when high bacterial counts were associated with histological evidence of an acute inflammatory process.

The incidence of pyelonephritis in potassium-depleted animals injected with enterococcus was three times that of simultaneously run controls (Table 3). Also, infections in tissues other than the kidney were much more numerous, and renal and extrarenal infections co-existed.

Growing animals on a potassium-free diet gain weight at a subnormal rate. Control animals in Group 4 were therefore pair-fed against potassium-depleted animals to determine if lack of an adequate caloric intake would alter susceptibility to infection.[17] The pair-fed controls gained an average of 46 gm., whereas other controls gained 72.9 gm. There was, however, no difference in susceptibility to enterococcus among the several control groups.

In both control and potassium-depleted animals, the nature and extent of infections in the kidney and other tissues requires special mention. Infection in the kidney was not visible grossly. Microscopically, there was no characteristic distribution, the cortex and medulla being involved in a random fashion, so that no specific relationship to the structural obstructive changes of potassium deficiency could be established. The infection consisted of small foci of interstitial exudation, predominantly polymorphonuclear, often with local tubular necrosis (Fig. 1-D). Brown-Brenn stains demonstrated the presence of Gram-positive cocci. In general, the pyelonephritis was relatively minor with little tendency toward extensive involvement of renal parenchyma.

Infection in other tissues was grossly visible. Mesothelial surfaces, such as the pleura and peritoneum, were thickened and dull and often coated with fibrinopurulent exudate. Fibrofatty tissues, such as mediastinum and retroperitoneum, were swollen and edematous. Histologically, the infection was suppurative (Fig. 1-B), frequently with marked necrosis. Although involvement of pleura and mediastinum was often widespread, infection was not seen in the lung parenchyma (Fig. 1-A). The pancreatitis was interstitial with regions of acinar necrosis (Fig. 1-C). The infective process tended to spread through the retroperitoneum, so that involvement of perinephric fat was not uncommon. Spleens from animals with well-established infectious disease showed proliferation of reticulo-endothelial elements and foci of polymorphonuclear cells occasionally with local necrosis. Examination of the endocardium with a dissecting microscope revealed no evidence of endocarditis. The infectious process in these sites tended to be extensive and progressive, which in about one quarter of the potassium-

depleted animals led to death. These findings are somewhat similar to those reported by Gray *et al.*[8] on staphylococcal-induced infections in mice. Their mortality was 15 per cent, and they found abscesses in kidney, heart, liver, intestinal mucosa, pancreas, and brain.

DISCUSSION

Milne *et al.*[11, 12] have suggested that there is a high incidence of pyelonephritis in patients with potassium depletion as a result of increased susceptibility of the kidney due to damage by potassium deficiency. Striking changes have been described in the nephropathy of potassium-depleted animals consisting of epithelial degeneration, necrosis, and proliferation of medullary collecting tubules, often leading to obstruction with dilatation proximally. Furthermore, Fourman *et al.*[6] observed prominent interstitial scarring which was progressive, even in the face of potassium repletion. Such lesions have not been seen in man. The usual finding is vacuolization of epithelial cells confined to the proximal convoluted tubules, the collecting system in most cases appearing normal.[14] In the absence of obstructive or other structural alterations, there is no obvious reason why degenerative changes in the proximal convolutions should enhance susceptibility to infection.

Relman and Schwartz[14] have recently reviewed the occurrence of pyelonephritis in reported cases of potassium depletion. In 47 cases of nephropathy caused by diarrhea, pyelonephritis was reported in 6 of 13 cases of primary aldosteronism. From this data they concluded that a predisposition to pyelonephritis was suggestive in the nephropathy associated with aldosteronism but unconvincing in diarrhea. In renal biopsies from patients with primary aldosteronism, interstitial scarring, glomerular hyalinisation, and tubular changes secondary to hypertension could be misinterpreted as evidence of pyelonephritis. More clinical observations must be compiled before definite conclusions can be drawn regarding the susceptibility of the potassium-depleted human kidney to infection.

Because of the marked differences in renal structural changes associated with potassium depletion in man and animals, interpretation of experimental data dealing with susceptibility to infection may not be directly applicable to humans. Moreover, it has been shown that renal scars, even when minute, enhance susceptibility of the kidney to infection in normal animals.[9, 10] Renal scars develop in the nephropathy of experimental potassium depletion and clinical aldosteronism and these lesions rather than potassium lack could be the chief factor causing infection. In order to minimize this in our animals, the degree of potassium depletion was of

sufficient severity to cause prominent structural alterations in the tubules with renal enlargement but without the development of significant interstitial fibrosis. In this manner, the effects of acute, severe potassium depletion upon susceptibility to infection could be studied without the complications of renal scarring and its secondary morphological changes.

It is not surprising that potassium-depleted rats and mice did not develop pyelonephritis after the intravenous injection of *E. coli*. Rocha[15] found that this organism produced no significant incidence of pyelonephritis in rats with severe injury following uric acid or mercuric chloride administration. In addition, *E. coli* failed to cause pyelonephritis in rats with vitamin D-induced nephrocalcinosis.[3]

Woods, et al.[16] have shown that rats with both acute and chronic nephropathy of potassium depletion have increased susceptibility to pyelonephritis produced by the intravenous administration of *E. coli*. Their strain of *E. coli* was highly virulent in rats, since it produced death or pyelonephritis in a large percentage of normal animals. When injected in small numbers, it is possible that this organism might reveal lesser degrees of susceptibility to infection than the strain of *E. coli* used in the present report.

In the present experiments, enterococcus produced a higher incidence of renal infection in potassium-depleted animals than in controls. Pyelonephritis was associated with infection elsewhere, the former being mild in comparison with the latter. The increased incidence of infection in potassium-depleted rats injected intravenously with enterococcus suggests that potassium deficiency may enhance susceptibility to some generalized infections. This is not unexpected since potassium is the principal intracellular cation and influences many cellular metabolic processes, possibly including those necessary for natural defense against infectious agents. Certain organic acids, including ketone bodies, have been shown to enhance the growth of bacteria in tissues. It is conceivable that the accumulation of such acids may be favored by a state of potassium depletion.

SUMMARY

E. coli failed to induce pyelonephritis in acutely potassium-depleted rats and mice. Enterococcus produced a higher incidence of generalized infection and pyelonephritis in potassium-deficient rats than in controls. Infections in extra-renal sites were extensive and often overwhelming, while those in the kidney were relatively mild. These data suggest that potassium depletion enhances generalized susceptibility to some infections with no primary effect on the kidney.

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ACID-SOLUBLE NUCLEOTIDES OF PENICILLIN-INDUCED PROTOPLASTS OF *ESCHERICHIA COLI***

The bactericidal action of penicillin is presently believed to result from alterations in nucleotide and peptide metabolism related to the structure of bacterial cell wall material. Park and Strominger have shown that in the penicillin-sensitive staphylococcus the drug causes the rapid accumulation of a group of uridine nucleotides.[13, 14] These compounds contain a hexosamine, acetylmuramic acid, and peptides of D-glutamic acid, L-lysine, and D- and L- alanine.[14] These substances are components of the staphylococcal cell wall and have been shown to be present in the same ratio in the nucleotides and in the cell wall.[14]

In gram negative bacteria such as *E. coli* penicillin is known to induce the development of osmotically unstable spherical forms which undergo rapid lysis.[8] Lederberg[5] and others[8] have recently demonstrated that a concentrated sucrose medium will prevent this lysis, and the stabilized round forms which develop have been designated protoplasts.[5, 6] They presumably represent a form of the bacterium with some alteration of cell wall structure. A polysaccharide present in the *E. coli* cell wall is known to contain muramic acid, glucosamine, alanine, diaminopimelic acid, glutamic acid, lysine, and glycine,[20] and it is this complex polysaccharide which appears to be altered by penicillin in inducing protoplast formation.[6, 10, 11, 17, 22] The accumulation of uridine nucleotides in the staphylococcus suggested that the change in cell wall properties in the *E. coli* protoplasts might also be accompanied by an alteration in nucleotide metabolism. This paper describes a study of the acid-soluble nucleotides and the nucleic acids of the penicillin protoplasts and the normal rod-shaped *E. coli* from which they are derived.

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METHODS

Culture of organism

Escherichia coli (ATCC 9637) was maintained on agar slants and used throughout.

Two culture media were employed. Medium E[22] contained inorganic salts and glucose. Medium A had the following composition:

Difco Bacto Yeast Extract	1.5 gm.
Difco Bacto Nutrient Broth	6.5 gm.
$MgSO_4 \cdot 7H_2O$	4.2 gm.
Medium E stock buffer[22]	20.0 ml.
Sucrose (commercial granulated sugar)	200 gm.
Glucose	5.0 gm.
Distilled water to make	1,000 ml.

The glucose and sucrose were autoclaved separately in concentrated solutions and then added to the complete medium.

All cultures were inoculated with 5 to 20 ml. of a late logarithmic phase culture in Medium E. Intact bacteria for nucleotide fractionation were grown in Medium A mixed with one-third volume of Medium E. These cultures were incubated at room temperature with shaking in three-liter bacterial culture flasks or in vigorously aerated eight-liter bottles and were harvested late in the logarithmic phase of growth.

Preparation of protoplasts

One volume of an overnight culture grown in Medium E at 37° was added to three volumes of Medium A supplemented with 1,000 units per milliliter of crystalline sodium or potassium penicillin G. The resulting mixture (2 to 8 liters) was incubated at room temperature either with shaking or aeration as in the normal bacteria cultures. Morphological changes (in gram or methylene blue stains) were almost identical with those described by Lederberg.[5,6] Incubation was continued until 80 to 90 per cent of the cells were transformed to rounded or slightly irregular three- to five-micron spheres.

Harvesting and separation of components

Intact bacteria or protoplasts were harvested using either a Sharples or conventional centrifuge and washed three times with a solution of 20 per cent sucrose in 0.2 per cent $MgSO_4$. (Stained slides showed no morphological change during these procedures.) Sufficient perchloric acid (PCA) was then added to give a volume four times that of the sedimented bacteria and a final concentration of 6 per cent. This mixture was stirred gently at 0° for 15 minutes. The supernate (acid-soluble fraction) was separated by centrifugation and neutralized to pH 7 with KOH. After standing at least one hour at 4°, the light green supernate was separated from the potassium perchlorate precipitate. The volume and optical density at 260 and 280 mμ were then measured.

After washing the material originally precipitated by perchloric acid with four volumes of cold 6 per cent PCA, the nucleic acids were dissolved by heating 30 to 45 minutes with 25 ml. of 6 per cent PCA in a boiling water bath. The protein which remained in the precipitate was then dissolved by gentle warming in 0.5 N NaOH.

Fractionation of acid-soluble nucleotides

The acid-soluble fraction was analyzed by gradient elution from Dowex-1 (formate) ion exchange resin by the procedure of Hurlbert *et al.*[4] A column 2 cm. in diameter

and 15 cm. in length was employed. The mixer volume varied from 400 to 800 ml., and the resin was eluted successively with two mixer volumes, of 4 N formic acid, and 0.2 N, 0.4 N, and 0.8 N ammonium formate in 4 N formic acid. The fractions comprising the principal ultraviolet absorption peaks were then pooled, and the optical density at 260 and 280 mμ was measured. If the peak had been eluted with ammonium formate, the nucleotides were adsorbed on charcoal and, after washing to remove adhering salt, were eluted with a mixture of ethanol and concentrated ammonium hydroxide. The pooled peaks were then evaporated to dryness under reduced pressure.

Determination of RNA, protein, and DPN

Protein was measured by the Folin reaction,[9] RNA by the orcinol method,[10] and DPN by reduction with alcohol dehydrogenase.[9] RNA determinations on the last PCA washing of the nucleic acids showed negligible interference from residual sucrose.

Characterization of nucleotides by paper chromatography

All chromatograms were run descending; and for analytical purposes Whatman number 1 paper was employed. Unhydrolyzed nucleotides were tentatively identified using n-butanol-acetic acid-water (2:1:1) and a propanol-ammonium sulfate solvent.[13] The latter was prepared by dissolving 600 gm. of ammonium sulfate in 1 liter of 0.1 M sodium phosphate buffer which had been adjusted to pH 6.8 with HCl and adding 20 ml. of n-propanol to the resulting solution. Fractions from the ion exchange column were further purified by chromatography on Whatman number 2 paper in butanol-acetic acid-water.

Nucleotides were hydrolyzed for eight hours in 6 N HCl in a sealed tube at 100° following which the HCl was removed by evaporation under reduced pressure. For subsequent chromatography, the following systems were employed: n-butanol-acetic acid-water (2:1:1) and (4:1:5),[15] phenol-water (4:1 with small volume dilute NH_4OH in tank), phenol-water (4:1),[13] 2,4-lutidine-water (3:2),[14] and methanol-pyridine-10 N HCl-water (80-10-2.5-17.5).[16] Phenol was distilled before use. Purines and pyrimidines were detected by their ultraviolet absorption and amino acids and hexosamines by spraying with a solution of 0.5 per cent ninhydrin and 1 per cent collidine in 95 per cent ethanol in a manner similar to the method of Levy and Chung.[7] Hexosamines were also detected separately by a modification[10] of the Elson-Morgan procedure of Partridge.[18]

Chromatograms of unknown solutions were run in the presence of appropriate standards. The standard amino acid mixtures of Levy and Chung were employed.[7] Since muramic acid was not available, the Rf of the farther moving of the hexosamines of the nucleotide fractions was measured relative to an alanine standard. The values so obtained were compared with figures given by Park[19] for the relative mobility of muramic acid in the same solvent systems. Agreement with the published values was within 8 per cent.

RESULTS AND DISCUSSION

The total acid-soluble nucleotide content of the penicillin-inhibited sensitive staphylococcus is approximately twice that of the normal organism.[20] This increase results from the accumulation of amino-acid-containing uridine nucleotides.[20] No comparable increase in total acid-soluble nucleotide

content was observed in the *E. coli* protoplasts. The relative amounts of nucleotides, RNA, and protein are given in the table and are almost identical in the normal *E. coli* and protoplasts.

Ultraviolet absorbing matarial was eluted from the ion exchange column throughout the entire range of formate ion concentration. In both normal

TABLE 1. RELATIVE AMOUNTS OF VARIOUS SUBSTRATES IN NORMAL *E. Coli* AND PENICILLIN PROTOPLASTS

	RNA	*Total acid-soluble nucleotides*	*DPN*	*Total of amino acid nucleotide fractions*
	Mgm. RNA (as AMP in orcinol reaction)/ mgm. protein	*O.D. 260 mμ x volume/ mgm. protein*	*O.D. 260 mμ x volume of peak/ O.D. 260 mμ x volume of total acid soluble fraction*	
Protoplasts (Mean ± standard deviation of mean)	0.230 ±0.046	0.390 ±0.047	0.318 ±0.053	0.032 ±0.015
Normal *E. coli* (Mean ± standard deviation of mean)	0.256 ±0.046	0.370 ±0.047	0.122 ±0.053	0.097 ±0.015
Difference (protoplasts—normal *E. coli*)	not significant*	not significant*	0.196 (p=0.01)	—0.065 (p<0.05)
Number of observations	15	13	8	5

* The minimum difference that could have been detected (would have been statistically significant) with the observed standard deviations are 0.098 and 0.120 for the RNA and nucleotide determinations respectively; (p=0.05). These would correspond to increases of the protoplasts of 38 and 32 per cent of the normal *E. coli* values.

E. coli and protoplasts the eluted fractions may be grouped into a series of comparable peaks. Two large peaks appeared in the early portion of the formic acid gradient section of the elution at positions compatible with DPN (peak 1) and AMP (peak 2).

On chromatography in butanol-acetic acid and propanol-ammonium sulfate, peak 1 yielded a component with the mobility of DPN. Its identity was confirmed by assay with alcohol dehydrogenase which demonstrated that 97 per cent of the ultraviolet absorption of a sample eluted from paper could be accounted for as DPN. An estimate of the DPN content relative to the total ultraviolet absorption of the acid-soluble fraction is given in

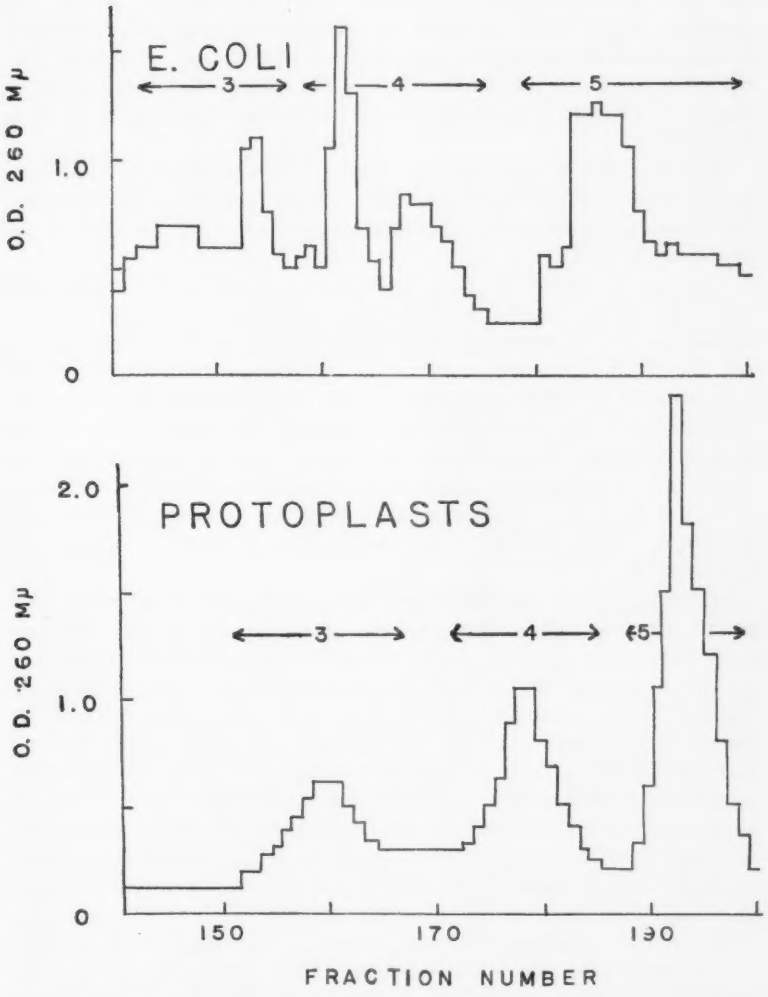

FIG. 1. Ion exchange chromatograms of the acid-soluble fraction of penicillin protoplasts and normal *E. coli*. The ultraviolet absorption of successive fractions from the column is plotted on the ordinate, but only the portion of the elution using 0.2 N ammonium formate in 4 N formic acid is shown in the figure. As indicated by the arrows, the eluted fractions have been grouped into a series of peaks, the composition of which is discussed in the text.

the table. This substance is the single nucleotide present in the greatest quantity. The relative amount in the protoplasts is increased to approximately 2.5 times that in the normal rod-shaped *E. coli.* A similar phenomenon apparently does not occur in the penicillin-inhibited sensitive staphylococcus,[20] and the mechanism of the increase in the protoplasts is not known.

Peak 2 on chromatography in the same solvent systems yielded one component with a mobility and spectrum identical to those of adenylic acid. The relative amount of this substance was approximately the same in the two bacterial forms.

The material eluted by 0.2 N ammonium formate in 4 N formic acid was grouped into a series of smaller, less well-defined peaks as shown in the diagrams of Figure 1. In the subsequent portions of the elution using 0.4 N and 0.8 N ammonium formate in 4 N formic acid, there appeared a series of small peaks containing amounts of ultraviolet absorbing material insufficient for characterization. Study of the peaks shown in the figure was directed towards detecting amino acids or hexosamines. To obtain sufficient quantities of material for characterization, 16-liter cultures of normal *E. coli* and 48-liter cultures of protoplasts were prepared, and peaks from three protoplast cultures and a single normal *E. coli* culture were then studied.

In the normal *E. coli,* peak 3 yielded five components on hydrolysis: uracil, muramic acid, glucosamine, glutamic acid, and glycine. Peak 4 gave these same substances and in addition alanine and diaminopimelic acid. A drawing of one of the chromatograms from this peak is shown in Figure 2. Peak 5 contained uracil and no amino acids or hexosamines. In the protoplasts peak 3 yielded uracil, muramic acid, glucosamine, glutamic acid, and glycine and was thus similar in composition to the corresponding peak in the normal *E. coli.* However, both peaks 4 and 5 gave uracil and no amino acids or hexosamines. The protoplasts thus appear to lack the nucleotide fraction containing diaminopimelic acid and alanine. Diaminopimelic acid was identified by its mobility and color in the methanol system of Ruhland[18] designed for its detection and by the characteristic yellow color which its spot was found to develop on standing 24 hours after chromatography in the lutidine solvent. Each protoplast fraction was chromatographed in one or both of these systems, and in no case was diaminopimelic acid found, though it was present in one of the normal *E. coli* fractions.

The quantitative composition of these nucleotide fractions was not established. With the techniques employed, it is possible to state only that the amount of uracil and amino acids seemed of the same order of magnitude.

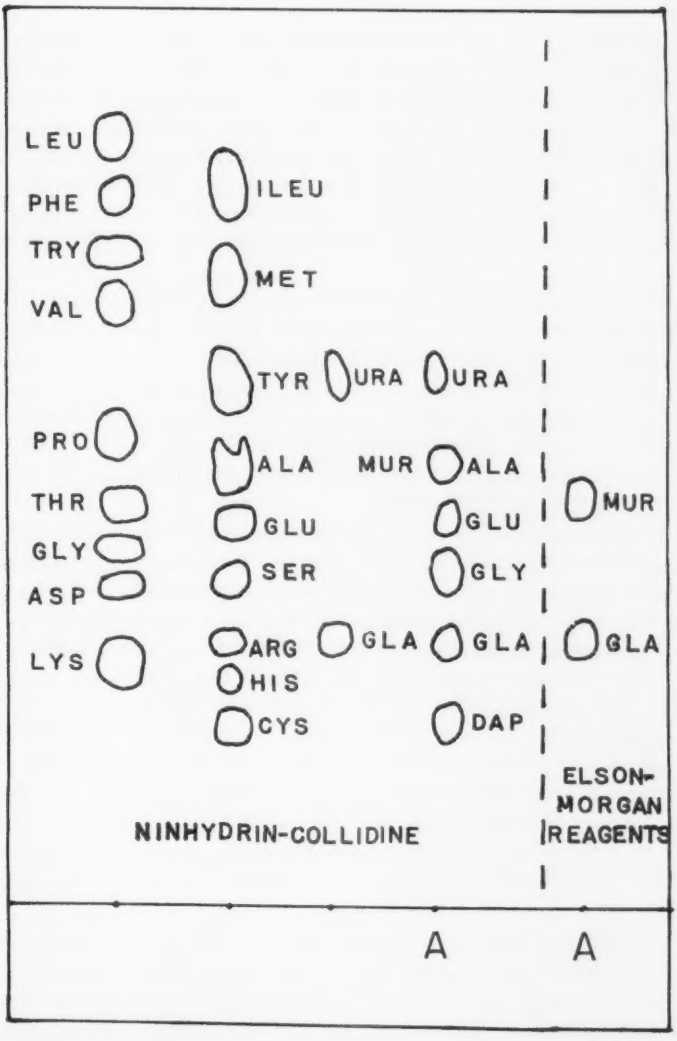

FIG. 2. Paper chromatogram of normal *E. coli* nucleotide fraction (peak 4 in Fig. 1.) run in n-butanol-acetic acid-water, 2:1:1. After removal from the tank the paper was cut along the broken line and the two portions treated as indicated. The hydrolyzed nucleotide solution was applied to the paper at the two points marked A. Abbreviations are as follows: Ala-alanine, Arg-arginine, Asp-aspartic acid, Cys-cystine, DAP-α,ε-diaminopimelic acid, Gla-D-glucosamine, Glu-glutamic acid, Gly-glycine, His-histidine, Ileu-isoleucine, Leu-leucine, Lys-lysine, Met-methionine, Mur-muramic acid, Phe-phenylalanine, Pro-proline, Ser-serine, Thr-threonine, Try-tryptophan, Tyr-tyrosine, Ura-uracil, Val-valine.

The molecular homogeneity of the fractions was not shown in these experiments; they merely demonstrated the association of the various components in the same ultraviolet absorption peaks. The presumption that the amino acids and hexosamines were part of an original nucleotide compound is not proved but seems warranted from the nature of the separation and extraction methods employed. The glucosamine seen in the chromatograms could have been derived from free glucosamine linked to a nucleotide or could have been a product of the cleavage of the ether linkage of muramic acid.

Isolation of these fractions suggests that in *E. coli* as in *S. aureus,* uridine nucleotides of a similar type play a role in formation of complex wall polysaccharide. After this work was completed, Strominger[21] reported the isolation from a mutant strain of *E. coli,* of a uridine diphosphate nucleotide containing muramic acid, alanine, diaminopimelic acid and glutamic acid. The probable relationship of this compound (which did not contain glycine) to the nucleotide fractions described in the present study remains to be demonstrated.

An estimate of the relative amounts of amino-acid-containing fractions in protoplasts and normal *E. coli* is given in the table. Because the compounds appear to be qualitatively different in the two cell forms, the significance of the somewhat greater amount in the normal bacteria is not clear. Strominger found the accumulation of uridine nucleotides in *S. aureus* resulted in a series of large peaks in the elution from the penicillin-treated bacteria.[20] The amount of nucleotide-linked hexosamine was very much greater in the penicillin-inhibited bacteria.[20] It is apparent both from the elution diagrams and the table that no comparable accumulation of peptide nucleotides occurs in the penicillin protoplasts of *E. coli.* The difference in amino acid composition, however, was found consistently in all cultures studied. These results are consistent with the recent demonstration of Nathenson and Strominger that penicillin inhibits incorporation of diaminopimelic acid into the cell wall of *E. coli.*[22]

The findings of the present study suggest that in *E. coli* penicillin affects incorporation of substances such as diaminopimelic acid into peptide nucleotides. In staphylococci a muramic acid pentapeptide nucleotide accumulates in the presence of penicillin.[13, 14] This has been interpreted as indicating that the antibiotic blocks the transfer of hexosamine-peptide groupings from this nucleotide to the cell wall.[14] In *E. coli,* on the other hand, penicillin seems to alter the incorporation of amino acid components into the peptide chain of the nucleotide. It might in this way interfere with formation of nucleotide precursors of cell wall material.

The lack of accumulation of peptide nucleotides in the penicillin protoplasts is in keeping with results in certain resistant gram-positive species. In these strains no accumulation of hexosamine nucleotides occurred even when the cultures were treated with bactericidal concentrations of penicillin.[30] The organisms studied were *S. faecalis* and a laboratory developed resistant strain of *S. aureus* which presumably did not produce penicillinase.[1] Although the mechanism underlying action of the drug in these organisms is not known, it would seem from the results in the *E. coli* protoplasts that cell wall formation may be altered without the accumulation of uridine nucleotides observed in sensitive strains of the staphylococcus.

These experiments do not definitely establish that penicillin interferes with formation of nucleotide precursors of the cell wall of *E. coli*. Although the results do suggest such a mechanism, naturally other interpretations are possible. For example, a pentapeptide nucleotide might be produced in the protoplasts and diffuse (through the altered cell wall) into the culture medium. Since no decrease was observed in the quantities of other component nucleotides or in total nucleotide content in the protoplasts, the alteration in permeability of the wall would necessarily have to be somewhat selective. However, the medium was not examined for nucleotides, and such a possibility cannot be definitely excluded. This limitation as well as the crude characterization of the nucleotide fractions and the complexity of the underlying biochemical mechanisms necessarily qualify any specific mechanism that might be proposed on the basis of the experiments described in this paper.

SUMMARY

The nucleic acids and soluble nucleotides of penicillin-induced protoplasts of *E. coli* have been compared with those of the normal rod-shaped organisms.

No appreciable difference was found in the RNA and total nucleotide content of the two bacterial forms. The DPN content of the protoplasts was approximately 2.5 times that of the normal *E. coli*.

A nucleotide fraction from the normal *E. coli* yielded on hydrolysis uracil, muramic acid, glucosamine, glutamic acid, and glycine. A second fraction contained all these components and in addition alanine and diaminopimelic acid.

A fraction from the protoplasts contained the same five substances as the first normal *E. coli* fraction. The peak which seemed to correspond with the fraction containing alanine and diaminopimelic acid, however,

yielded uracil and no amino acids. The amino-acid-containing nucleotide fractions were not increased in the protoplasts compared with the control normal *E. coli.*

The findings suggest that in *E. coli,* penicillin may alter incorporation of components such as diaminopimelic acid into nucleotide precursors of the cell wall.

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EXPERIMENTAL PYELONEPHRITIS

V. SUSCEPTIBILITY OF RATS TO HEMATOGENOUS PYELONEPHRITIS FOLLOWING CHEMICAL INJURY OF THE KIDNEYS†

Renal infection rarely occurs following the intravenous injection of Gram-negative bacilli into normal animals. If, however, the kidney has been damaged by either direct trauma or ureteral ligation, an increased susceptibility to infection can be demonstrated.[2, 4] In this regard, studies have shown that the location of the site of injury within the kidney is important in determining alterations of host resistance. While multiple cortical lesions did not predispose the kidney to hematogenous infection, a single medullary scar resulted in anatomical and functional changes which permitted the multiplication of bacteria and resultant infection in the involved segment of tissue.[8]

This differential effect of localized injury suggested a study of several chemicals which are known to damage particular segments of the nephron. Mercuric chloride, which primarily causes necrosis of cells of the proximal convoluted tubules[10] and not the collecting tubules and a sulfonamide derivative, sodium N^4-acetyl-sulfathiazole, which produces along with some cortical damage obstruction of the collecting tubules by intraluminal crystallization and precipitation[1, 6] were administered to rats prior to bacterial injection. The results obtained indicate that while injury to the nephron excluding the collecting system did not affect susceptibility to hematogenous infection, intrarenal obstruction at the level of the larger collecting tubules did have that effect.

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FIG. 1. Microscopic illustration of the effect of mercuric chloride on the rat kidney. Observe the tortuous dilated tubules, with severe degenerative changes of tubular cells. There is also interstitial edema and round cell infiltration. (Hematoxylin-eosin x 170)

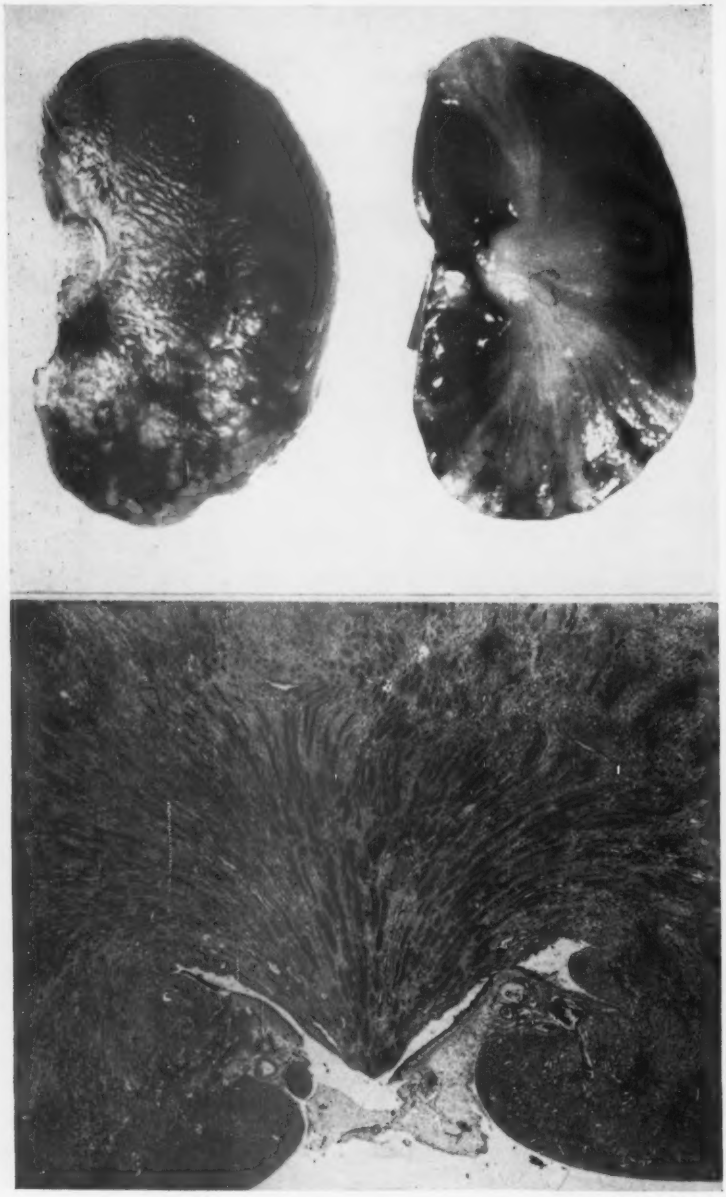

FIG. 2. Gross appearance of an infected kidney in a rat which received sodium N¹-acetyl-sulfathiazole followed by the inoculation of *E. coli*. Observe the multiple confluent abscesses mainly distributed at the lower pole. Cut section illustrates the distribution of infection radiating from the papillary zone to both poles.

FIG. 3. Papillary zone of an infected kidney in a rat which received sodium N¹-acetyl-sulfathiazole followed by the inoculation of *E. coli*. Notice the dilatation of collecting tubules; many of them packed with polymorphonuclear leukocytes. (Hematoxylin-eosin x 10)

MATERIALS AND METHODS

Animals. White male Sprague-Dawley strain rats, weighing 200 to 300 gm. were used.

Production of renal damage. The mercuric chloride and the sodium N^4-acetyl-sulfathiazole were given in a dose and manner previously shown to produce consistent renal damage in rats.[5, 6] Mercuric chloride was prepared in sterile distilled water so that each 0.2 ml. contained 1 mg. of drug. A dose of 1 mg. was given intramuscularly every day for four consecutive days. Sodium N^4-acetyl-sulfathiazole was similarly prepared under sterile conditions, the final concentration being 100 mg. per ml. A single intraperitoneal injection of 500 mg./kg. body weight was given.

Bacteria. The characteristics of the strain of *E. coli* used have been described in detail elsewhere.[4] The bacteria were incubated in beef heart infusion broth at 37° C. for four hours, and 0.5 ml. of a 4-hour culture was injected into the tail vein of each rat. Ten-fold dilutions in nutrient broth were incubated in agar pour plates to enumerate each inoculum. The average injection contained 150 to 200 millions of bacteria.

Removal of kidneys. Following pentobarbital anesthesia, the kidneys were removed under sterile conditions. After macroscopic examination, they were placed in a "Ten Broeck" grinder, or a tube of a "Precision" homogenizer and ground in 9 ml. of nutrient broth until a smooth suspension was obtained. This represented the 10^{-1} dilution. Subsequent ten-fold dilutions were made in the same medium, and pour plates were obtained from 10^{-2} and 10^{-8} dilutions. Plates were incubated for at least 48 hours, and the number of colonies recorded. When the plate containing the greatest dilution had too many colonies to be counted, the number was recorded as greater than 1,000.

EXPERIMENTAL

Susceptibility to infection after renal damage with mercuric chloride. The 20 rats in this experiment received an intravenous inoculation of *E. coli* one to two weeks after mercuric chloride administration and were sacrificed one week later. Only one of the 40 kidneys had 10^4 bacteria, the rest having from 0 to 10^3, which is the number to be expected in normal kidneys one week after intravenous injection of this inoculum.[4] It seems possible to conclude, then, that the kidney damage induced by mercuric chloride does not enhance renal susceptibility to hematogenous infection.

Susceptibility to infection after renal damage with sodium N^4-acetyl-sulfathiazole. Bacteriological examination of the kidneys was performed one week after the intravenous injection of *E. coli* into rats which had received sodium N^4-acetyl-sulfathiazole one to six weeks previously. As indicated in Table 1, kidney damage with sodium N^4-acetyl-sulfathiazole does enhance the susceptibility to infection, but susceptibility rate decreases as the time interval between kidney damage by the drug and bacterial inoculation lengthens.

Persistence of infection. A group of 34 rats was given sodium N^4-acetyl-sulfathiazole and then one week later *E. coli* by the intravenous route. At

intervals for six weeks after the inoculation, some of the animals were sacrificed and bacteriological and pathological examinations of the kidneys were performed. As indicated in Table 2, there is a tendency for spontaneous remission of the infection with time.

Test for occurrence of spontaneous infection. Mercuric chloride was given to eight rats and was not followed by a bacterial inoculation. The rats

TABLE 1. INCIDENCE OF PYELONEPHRITIS RESULTING FROM INJECTION OF E. COLI INTO RATS WITH RENAL DAMAGE CAUSED BY SODIUM N⁴-ACETYL-SULFATHIAZOLE

Time (weeks)[1]	*No. animals*	*No. animals infected*[2]
2	12	10
4	9	5
6	10	2

[1] Interval between N⁴-acetyl-sulfathiazole administration and bacterial injection.
[2] Animals with more than 100,000 bacteria per kidney.

TABLE 2. PERSISTENCE OF PYELONEPHRITIS IN ANIMALS WITH RENAL DAMAGE CAUSED BY SODIUM N⁴-ACETYL-SULFATHIAZOLE

Time (weeks)[1]	*No. animals*	*No. animals infected*[2]
2	15	12
4	8	7
6	11	4
	34	23

[1] Interval between bacterial injection and kidney examination.
[2] Animals with more than 100,000 bacteria per kidney.

were divided into two groups. One group was examined two weeks after and the other, three weeks after the mercuric chloride injection. In all, bacteriologic studies proved to be negative. Similarly, sodium N⁴-acetyl-sulfathiazole was given to 21 rats, and was not followed by bacterial inoculation. The time interval between the injections of the sulfonamide and cultural and pathological examinations was one week in two rats, two weeks in two, three weeks in four, four weeks in four, five weeks in five, and six weeks in four. Cultures of the kidneys and the bladder urine were sterile in every instance.

Macroscopic and microscopic appearance of the kidneys. The gross appearance of kidneys after injury with mercuric chloride was one of diffuse swelling, without evidence of infection. During the acute phase of intoxication (one week) damage to the convoluted tubules was evident, with some dilatation, interstitial edema, round cell infiltration, and tubular cell destruction (Fig. 1). The collecting system was usually not affected.

The presence of infection in rats which received sodium N⁴-acetyl-sulfathiazole followed by intravenous inoculation of *E. coli* could not always be determined by simple inspection. In both control and inoculated animals the surfaces of the kidneys were sprinkled with many small, white, slightly raised spots suggesting infection, but, in actuality, these were deposits of the sulfonamide. However, in certain cases clusters of yellow, slightly raised areas of purulent exudate, occasionally coalescent, were visible beneath the capsule. In those cases, as seen in Figure 2, infection could be recognized macroscopically. When the animal was sacrificed three to six weeks after *E. coli* administration, the kidneys appeared small in size and heavily scarred. The histological appearance of the kidneys of rats which received only sodium N⁴-acetyl-sulfathiazole has been described elsewhere.[8] In some instances of infection, there was a much larger degree of kidney destruction than that produced by the sulfonamide alone. In those instances, there were diffusely scattered abscesses and collections of polymorphonuclear leukocytes both within and surrounding the dilated tubules (Fig. 3).

DISCUSSION

There seems to be a significant difference between the susceptibility of rats to pyelonephritis after kidney damage with sodium N⁴-acetyl-sulfathiazole and that following administration of mercuric chloride. The reasons for this difference are probably multiple, but we believe one of them is the difference in site of kidney damage produced by these two drugs. In the dosage used here damage caused by mercuric chloride is confined to the convoluted tubules of the cortical zone, and no remarkable alteration could be detected at the medullary zone. On the other hand, sodium N⁴-acetyl-sulfathiazole was found to deposit in the collecting tubules, in the medulla and papilla, with the result that there was a considerable degree of intrarenal obstruction throughout the extent of the tubule. Mechanical injury of this area has already been shown to enhance susceptibility to infection. The results obtained in these experiments in rats seem to agree with those previously obtained by thermal injury of the kidneys of rabbits.[8]

Animals given the sulfonamide did not develop infection spontaneously. It seems possible that the descriptions of 'pyelonephritis' secondary to the

use of sulfonamides as reported by other investigators[5,7] were based only on the observations of an inflammatory reaction noted in the kidneys of those animals. However, the presence of inflammatory cells does not necessarily mean infection. In this study no bacterial growth was demonstrable in 21 animals examined at varying intervals up to six weeks after the administration of the sulfonamide.

SUMMARY

After rats were given mercuric chloride, kidney lesions developed which were confined primarily to the cortical zone. Animals with this form of renal injury did not exhibit increased susceptibility to infection following intravenous administration of *E. coli*. In contrast, the renal lesion secondary to sodium N⁴-acetyl-sulfathiazole, which is mainly in the collecting tubules, and which results in intrarenal obstruction, was found to enhance susceptibility to this kind of infection. The location of the damage appears to be an important factor.

Spontaneous infection did not tend to develop in the kidneys of animals treated with the sulfonamide compound alone. The histology of these kidneys was compatible with the diagnosis of pyelonephritis despite the inability to demonstrate bacterial infection. One must conclude therefore that all histological pyelonephritis need not result from bacterial infection. The suggestion is offered that previous descriptions of 'pyelonephritis' following this treatment were justified only by the demonstration of cellular infiltrates.

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NEUROLOGICAL STUDY UNIT

October 13, 1959

HEMIBALLISMUS, RIGIDITY, AND THE BASAL GANGLIA.* By J. Purdon-Martin, National Hospital, Queen Square, London.

Renewed interest in the clinical-pathological approach to basal ganglia disease has been occasioned by the extension of surgical procedures to this area. Anatomically, the internal capsule separates the globus pallidus and striatum laterally from the more medial subthalamic nucleus (corpus Luysii) and substantia nigra. Fiber connections show these ganglia to be grouped with the globus pallidus at the center, receiving fibers from the striatum and substantia nigra and having a two-way connection with the corpus Luysii. Two longer afferent pathways leave the globus pallidus via the ansa lenticularis and fasciculus lenticularis to end in the ventral thalamus. Afferent fibers come to these structures from other parts of the brain, those of cerebellar origin being the most important.

Evidence from clinical-pathological observation and from animal experiments indicates that tissue destruction limited to the corpus Luysii gives rise to a marked hemichorea, or hemiballismus, but with little reduction in muscle tone. Within limits, the underlying capacity for voluntary movement (such as touching index finger to nose) remains intact. Hemiballismus can be abolished by a surgical lesion placed either in the globus pallidus or in the ventral thalamus (pallido-fugal block). Again, in cases of idiopathic Parkinson's disease or postencephalitic parkinsonism in which muscular rigidity was a sole or major involvement, the cells of the substantia nigra are damaged or completely destroyed, and surgical evidence is accumulating that operative lesions in the globus pallidus abolish rigidity in these cases.

A destructive lesion cannot give rise to "positive" symptoms; the irregular impulses which enter the motor system must arise from and be transmitted along fibers that remain intact. Any pathological disturbance in the basal ganglia, therefore, must be considered in relation to the group as a whole. Hemiballismus and the muscular rigidity of parkinsonism interfere with voluntary movement by maintaining a continuous and excessive muscular activity. The anatomical arrangement of the basal ganglia suggests that the group acts through the globus pallidus. A lesion here or among its afferent fibers in the fascicularis lenticularis abolishes the positive symptoms and it seems that the responsible impulses must emanate from the globus pallidus. The corpus Luysii seems to act as a moderator of impulses from the globus pallidus. Destruction of the substantia nigra, with the corpus Luysii intact, leads to excessive muscular tone and rigidity; thus the substantia nigra would seem to exert under normal circumstances a direct or indirect inhibition upon the activity of the globus pallidus. The striatum as well seems to exert an inhibitory control.

The physiological conditions under which the mechanism for muscular activity contained in these structures is called into play are unknown. When

* See Purdon-Martin, J.: The functions of the basal ganglia. *Lancet*, 1959, *1*, 999.

its action is blocked, no defect in normal function is apparent. It is suggested that the dynamic control of such a mechanism would hold it ready to be brought into operation in special circumstances.

D. M. B.

October 14, 1959

NEURO-OPHTHALMOLOGY. By David Cogan, Boston, Mass.

Dr. Cogan presented brief case histories of a number of patients suffering from brain lesions. In these patients visual difficulties were accompanied by other neurological symptoms. For lesions in the parietal and temporal lobes the signs and symptoms produced could be placed in three general categories:

1. Signs and symptoms associated with lesions of either side.
   (a) Opticokinetic response (deviation to the side of the lesion)
   (b) Deviation of eyes with closure of lids (deviation to the side opposite the lesion)
   (c) Astereognosia and metamorphopsia
   (d) Confusion and inability to fix
   (e) Photopsia, hallucinations and illusions
   (f) Convulsive episodes
   (g) Brain stem compression and herniation.

   It is worth noting that the opticokinetic response is independent of hemianopsia. In normal people the response is always symmetrical, although the absolute response varies from person to person.
2. Signs and symptoms associated with the dominant hemisphere.
   (a) Alexia
   (b) Aphasia
   (c) Acalculia
   (d) Finger agnosia (body agnosia in general)
   (e) Right-left confusion.
3. Signs and symptoms involving the nondominant hemisphere.
   (a) Topographic agnosia
   (b) Constructional apraxia
   (c) Dressing apraxia
   (d) Neglect of left side
   (e) Auto accidents (?).

   Dr. Cogan was not certain that 3(e) above should be put in definitive form, but in his experience patients in category 3 had shown a distinct tendency to have automobile accidents.

An additional category was concerned with lesions in the occipital region. Some of the characteristics of such lesions could be described by the following list:

   (a) Hemianopsia
   (b) Photopsia and hallucinations

(c) Pain in homolateral eye (?)
(d) Denial of blindness
(e) Los of visual imagery
(f) Convulsive deviation of eyes
(g) Brain stem signs
(h) Absence of other neurological signs.

Of these symptoms (h) was regarded as most characteristic, although exceptions could always be found.

S. J. F.

ZOOLOGICAL JOURNAL CLUB

October 14, 1959

DISTRIBUTION OF THE BATHYPELAGIC FISHES OF THE GENUS *MELAMPHAES* IN THE EASTERN PACIFIC OCEAN. By Alfred W. Ebeling, Scripps Institute of Oceanography, La Jolla, California.

Early concepts of the zoogeography of the bathypelagic organisms, those found below 200-300 meters in the ocean, were based on the theory of widespread distribution, due to the uniformity of environmental conditions and the lack of large physical barriers in this realm. Recent studies, however, demonstrate that many bathypelagic species display a localization in specific geographical areas.

The distribution of individual species of the genus *Melamphaes,* consisting of 19 presently described species, generally conforms to the geographical location of distinct water masses in the Pacific Ocean. Water masses are usually delimited by distinctive temperature and salinity characteristics.

Data for one species, *M. aconthomus,* as well as inferences from other studies, suggest that young and larval stages are more closely confined to their respective water masses than are the adults, which may wander beyond the limits of the breeding population. Adult fish are also commonly capable of diurnal migrations of several hundred meters, whereas the young ontogenetic stages are thought to be restricted to a narrower range of depths nearer the surface. Although physico-chemical features of water masses may be important in directly limiting the distribution of young stages, the biological patterns associated with water masses are believed more important as isolating mechanisms between species. Species of larger size, four to six inches, are associated with regions or water masses of relatively high photosynthetic activity, while dwarf species are generally found in the less productive areas. It is suggested that low food supply or different types of food in the less productive regions may have favored natural selection for these morphological differences. Further evidence for this theory comes from a single species found in two separate areas of different productivities; again the larger individuals are associated with the area of higher productivity.

W. PEARCY

ANATOMY SEMINAR

October 20, 1959

PROBLEMS OF BIOASSAY OF HUMAN URINARY PITUITARY GONADOTROPHIN. By R. Borth, University Clinic of Gynecology and Obstetrics, Geneva, Switzerland.

There are four essential problems which must be considered in the design of an effective hormone assay method: one, technique; two, reliability, which includes specificity, accuracy, precision, and sensitivity (in bioassay, specificity often has meaning only in terms of the end point used, since many biologically active compounds, e.g., the luteinizing hormone, have not been chemically identified); three, practicability, under which are subsumed speed, expenditure, and skill; four, sources of error.

To achieve a good bioassay, it is essential to secure a dosage-response curve for both the unknown and a standard. The standard must be chosen with care so that it is similar to the unknown extract. If the dosage-response curve of the unknown is not parallel to that of the standard, the two preparations are dissimilar, and the assay cannot be evaluated. Even with parallel dose-response curves, two preparations may be biologically dissimilar as evidenced by a variation in their relative potency when different end-points are used. By the latter criterion, some degree of dissimilarity has been demonstrated between gonadotrophin preparations obtained by various authors from human nonpregnancy urine.

There may be several resasons why two preparations are biologically dissimilar. Two or more active factors may be present in unequal ratios, and the two end-points used may be differently sensitive to such mixtures. A difference in inactive material affecting absorption, inactivation and transport may manifest itself differently, with regard to the time-concentration curve in the target organ, in two assay methods. Differences in toxicity may affect two end-points differently. Two responses may be differently sensitive to interfering substances such as synergists, antagonists, toxin.

In view of these possibilities, it would be unwise to interpret evidence of dissimilarity of gonadotrophin preparations too rashly only in terms of FSH/LH ratios.

R. BORTH

ZOOLOGICAL JOURNAL CLUB

October 28, 1959

A COMBINED BIOCHEMICAL, HISTOCHEMICAL AND ELECTRON MICROSCOPIC STUDY OF OXIDATIVE ENZYMES. By Russell J. Barrnett, Yale University.

Recent studies on the localization of oxidative enzymes throw new light on the problem of relating function and structure in biological systems. The succinic dehydrogenase system of mammalian heart muscle has been localized by using potassium talurite as an electron acceptor. Following the reaction (as the substrate is oxidized and the reagent reduced by an enzymatic transfer of electrons), crystalline and particulate precipitates can

be detected with the electron microscope and are found to occur predominantly in mitochondria, the crystals being closely associated with, and oriented parallel to the cristae. Similar studies using tetrazolium salts as electron acceptors (as nitro- and iodonitro- BT), and high resolution electron microscopy, reveal that the final products of the reaction (formazans) are deposited on cristae in various parts of mitochondria.

Evidence of another type comes from electron microscopy of an isolated and purified preparation containing cytochromes, succinic dehydrogenase, and DPNH-oxidase isolated from heart muscle. The preparation was obtained by homogenization and repeated extraction with distilled water, coupled with centrifugation and precipitation with salt solutions. Enzyme systems that would still oxidize succinate or DPNH were associated with membranous structures in such preparations. Treatment with deoxycholate resulted in the removal of cytochrome c from the system; other absorption curves were for the most part unaffected. Electron micrographs of the remaining enzyme-containing system revealed paired membranous structures.

Thus, evidence favors the hypothesis that terminal oxidative enzymes not only are found in mitochondria, but also are intimately related to membranous structures therein.

PHYSIOLOGY SEMINAR

November 4, 1959

ANALYSIS OF CEREBRAL PERFUSATE FOLLOWING STIMULATION OF THE CENTRAL NERVOUS SYSTEM. By Loring F. Chapman, Assistant Professor of Physiology, Cornell Medical College, New York City.

The presence of a polypeptide responsible for local vasomotor control has been demonstrated by past investigations, notably those of Keele *et al.*, who showed that a polypeptide was present in blister fluid, in fluid collected from painful joints, and in inflammatory pleural fluid. Ostfeld *et al.* demonstrated that a similar substance is present in tissue fluid removed from regions of local scalp tenderness during vascular headaches of migraine type. Hilton and Lewis described a proteolytic enzyme present in saliva and in saline perfusate of salivary gland; this enzyme formed vasodilator polypeptides when incubated with plasma proteins. These authors observed increased amounts of enzyme during heightened metabolic activity of the gland and concluded that the enzyme and polypeptides are responsible for local vasomotor control in the salivary gland. The properties of the polypeptides corresponded to those of "bradykinin," the name given by Roche é Silva, Beraldo, and Rosenfeld to substances produced on incubation of certain snake venoms or trypsin with serum, plasma, or plasma globulin. These authors reported that when such incubated mixtures were applied to smooth muscle (isolated rat uterus or guinea pig ileum), delayed, slow, and sustained contractions occurred.

These observations led to the study of the biochemical aspects of cerebrospinal fluid in clinical cases, from which it was concluded that increased amounts of "protease" and vasodilator polypeptides in cerebrospinal fluid are associated with (1) active inflammatory or degenerative disease of the

central nervous system, (2) vascular headache of migraine type, associated with sustained vasodilation, (3) prolonged noxious stimulation or pain (sustained excitation within the central nervous system), and (4) chronic schizophrenia. It is suggested that the protease-polypeptide system is implicated in local vasomotor control within the central nervous system, and when in excess the components of the system may be related to disease.

In animal studies carried out with Ramos and Corrado in Brazil and with Symmes at Yale University, a system was employed in which a polyethylene cannula is inserted into the lateral ventricle of cats and another into the cisterna magna or into the sub-arachnoid space. Solution can thus be flushed in and out with ease. In cats anaesthetized with Nembutal and so cannulated, the sciatic nerve was stimulated. In other experiments the cats were curarized and maintained on artificial respiration. Samples of cerebrospinal fluid were collected at 10-minute intervals and assayed. The samples exhibiting the greatest "protease" activity appeared approximately 15 minutes after sciatic stimulation. After 30 minutes the cerebrospinal fluid again exhibited the normal absence of protease activity. At the end of the experiment the cannula was perfused with dye in order to make a more detailed inspection of the area studied. In other experiments chronic cannulae were implanted in the lateral ventricle and in the sub-arachnoid space overlying the cortex.

This perfusion technique offers promise in future work, since it gives one the opportunity to study secondary biochemical and pharmacological effects of drugs administered peripherally. It allows the administration of drugs directly into the cerebrospinal fluid over a long time interval. In chronic preparations, the effects of sleep deprivation, for example, could easily be studied. The technique offers a useful research tool in the investigation of drug penetration into brain tissue, the short and long term effects of drugs on brain metabolism and function, and of metabolites released into the cerebrospinal fluid during stress.

G. VIRGINIA UPTON

JOHN PUNNETT PETERS MEMORIAL LECTURE

November 17, 1959

THE CLIMATE FOR THE CULTIVATION OF CLINICAL RESEARCH. By Maurice B. Strauss, Boston University and Tufts University Schools of Medicine, Boston, Massachusetts.

John Punnett Peters believed that the special province of the clinical investigator was the study of disturbances created by nature in man. Fully aware that such research was more difficult than animal or test-tube investigation, he did not shrink from it and his work was never far removed from the patient. Two trends, both alien to Dr. Peters' creed, are now visible on the contemporary horizon—one, the divorce of the medical scientist from the bedside, and the other, a preoccupation with mathematical, biochemical, and biophysical techniques. Neither is new. The 17th century, during which medical thought was oriented to the exact sciences, saw the rise of iatromathematical, iatrochemical, and iatrophysical schools (translate biomathematical, biochemical, etc.). Significant discoveries were made but the pre-

occupation of the discoverers was with hypotheses: each school believed that all disease could ultimately be explained in terms of a single unified system —mathematical, chemical, or physical. The study of the patient was neglected and medicine suffered while physicians indulged in violent polemics. The inevitable reaction was accompanied by disinterest and hostility to the really important discoveries that had been made. It required Claude Bernard in the mid-19th century to reorient medicine in the direction of science. He made it abundantly clear that although there was but one order of nature, there were many techniques with which it might be studied. Each had its own problem and its own point of view, and confusing these could only lead scientific investigation astray. The complexity of the biological sciences, necessitating the help of all the other sciences, often caused increased confusion.

This confusion, in part, stems from a metaphysical assumption widely accepted in the 17th century and still prevalent today among biological scientists although long since rejected by physicists—the assumption that the properties of a whole system can be fully deduced from information about the behavior of its isolated parts. This assumption neglects the fact of organization, well recognized by the physicist who knows that an electron is only an aspect of a total situation, the major part of which is the rest of the apparatus, and that the quantum theory has clearly demonstrated that situations must be encountered as wholes. Isolation of parts produces artefacts not present in nature.

Until the biological scientist rejects this ancient but false assumption he will not be aware of the fact that the terms "more basic," "more fundamental," and the like have no meaning when applied to one or another division of science and that there is no "higher" and no "lower" among the disciplines employed in medical research.

Clinical investigation is arduous. It is not for those who suffer from the spiritual distemper of our time—the desire to make a "fast buck." It may not reward its disciples with a succession of papers turned out with clock-work regularity. It may not speedily win them research grants or academic promotion. However, it cannot be denigrated on the basis that it is concerned with a different level of organization than biochemical or biophysical research. The clinical investigator can repeat with Dr. Peters one of his favorite quotations: "The proper study of mankind is man."

M. R. STRAUSS

THE PHYSIOPATHOLOGY OF CANCER. 2nd ed. Freddy Homburger, Ed., with 32 authors. New York, Hoeber-Harper, 1959. xix, 1,180 pp., illus. $33.00.

This revised and enlarged edition of the original encyclopedic work on cancer is an important treatise consisting of 28 chapters by 32 contributors. The essayists are all leading cancer investigators, including one from the Chester Beatty Research Institute, London, England.

The Physiopathology of Cancer is divided into four sections: (I) Biology, with chapters on (1) experimental cutaneous carcinoma, (2) pulmonary tumors in mice, (3) mammary tumors in mice, (4) tumors of the liver in rats, (5) genetics of neoplasia, (6) hormonal factors in experimental carcinogenesis, (7) virus induced tumors and the virus theory of cancer, (8) transplantable tumors, (9) heterologous transplantation, (10) leukemia, and (11) tissue culture in the study of malignancy; (II) Chemistry and Physics, including chapters on (12) nutrition and cancer, (13) chemical and genetic mechanisms of carcinogenesis—mode of action, (14) biological alkylating agents, (15) chemistry of cancer tissue, (16) nucleoproteins and cancer, (17) enzymes and cancer, (18) cancer cytochemistry and histochemistry, (19) experimental cancer chemotherapy, and (20) radiation and cancer; (III) Clinical Investigation with chapters on (21) natural history of neoplastic disease, (22) steroid hormone metabolism in cancer, (23) clinical investigation in cancer, (24) environmental cancer, and smoking and lung cancer, and (25) statistical studies in cancer; and (IV) Practical Applications with three final chapters on (26) clinical cancer chemotherapy, (27) applied exfoliative cytology, and (28) applied radiation therapy. This monumental work contains something of interest for investigators and clinicians dealing with practically every phase of cancer from the experimental to the clinical syndrome.

Homburger's revised work presents a thorough cross-disciplinary analysis of cancer research and clinical observations. This book represents a truly great service to the whole field of cancer. Both the factual essays and the lengthy *and complete bibliographical citations* brilliantly codify the vast literature on this rapidly expanding subject. Every chapter deserves careful reading, for each provides well-documented evidence and well-thought-out perspectives.

The book is rightfully dedicated to the late Dr. Francisco Duran-Reynals (1899-1958) whose life's work stands as an erect posture of scientific investigation on the cancer problem.

JOSEPH THOMAS VELARDO

RADIATION BIOLOGY AND CANCER. A collection of papers presented at the Twelfth Annual Symposium on Fundamental Cancer Research, 1958. Austin, University of Texas Press, 1959. 493 pp. $8.50.

This volume represents a collection of papers presented at the Twelfth Annual Symposium on Fundamental Cancer Research, 1958, held under the auspices of the University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas. Some 44 authors presented 34 papers.

What has evolved is a survey of radiation biology as it applies to the field of cancer which is a close approximation to a textbook on the subject. There is also a section on the medical applications of radiation which includes the experience of Gilbert H. Fletcher in the treatment of 1,500 cases of various malignant tumors with Cobalt-60 teletherapy and a 22-MEV Betatron.

The major effort deals with carcinogenesis, and the conference committee had gathered together a major proportion of the leading authorities in the field. Not the least appreciated was the presentation of the present direction of their thinking even if in the formative stage. This was augmented by inclusion of the free discussion which followed the papers. With a collection of authors which include Jacob Furth ("Radiation neoplasia and endocrine systems"), Leon O. Jacobson ("Hematological effects of irradiation"), Henry S. Kaplan ("Radiation-induced leukemia in mice: A progress report"), Arthur Kirschbaum* ("Relation of radiation carcinogenesis to carcinogenesis induced by other means"), Walter J. Burdette ("Mutagenesis and carcinogenesis"), and others, one hesitates to single out any particular article for fear that the reader might gain the impression that the many others are not of the same high quality. Stanfield Rogers presents a summary of his provocative work on the mechanism of action of ultraviolet irradiation in initiating tumors in the lung tissue of mice. The influence of a number of factors such as radiation intensity, temperature, and state of the cells in their mitotic cycle are considered. He concludes that initiation of these pulmonary tumors is most probably due to chromosomal change. There are several reports on human material. One of these, by William C. Moloney, on "Induction of leukemia in man by radiation" is authoritative and properly circumspect. He notes the relative lack of chronic lymphatic leukemia in exposed groups.

Many classical references necessarily of recent date in these rapidly developing fields are included in the bibliographies. This volume is highly recommended.

MORTON M. KLIGERMAN

* Died May 28, 1958.

IMMUNITY AND VIRUS INFECTION. Victor A. Najjar, Ed. New York, John Wiley and Sons, 1959. 262 pp. $10.50.

Although the title might suggest that the scope of this volume is rather limited, such is far from being the case. The twenty papers, which were presented as a symposium at the Vanderbilt University School of Medicine in May 1958, cover a broad range from theoretical aspects of antibody formation to some of the practical aspects of immune mechanisms not only in the field of virus infections, but also in a number of other fields such as allergy, hypersensitivity, immune tolerance, and tissue transplantation. Each paper is written by an outstanding authority in his field. Background material of most of the subjects is well reviewed, and current concepts and observations are presented in an interesting and often provocative manner. Even the more theoretical and technical aspects of antibody production and measurement and the mechanisms of immune reactions are

written with such clarity that readers who are not familiar with the field should have little or no difficulty understanding the material presented.

Of particular current interest are the final three papers which deal with immunization against poliomyelitis. Dr. Albert Sabin presents a clear picture of present concepts of the pathogenesis of poliomyelitis (documented by careful investigation) which have led to the development of an attenuated live virus vaccine. Dr. John R. Paul *et al.* discuss use of Dr. Sabin's vaccine under conditions of experimental epidemiology and Dr. John P. Fox concludes with observations on the vaccine under limited field conditions. All three papers are concerned with the types of immunity conferred by natural vs. artificial means and their roles in the pathogenesis and epidemiology of the clinical disease.

The entire book is timely and stimulating in providing basic facts and suggesting areas for further research. It should provide an excellent reference for students and investigators in the basic sciences and clinical research.

ROBERT W. MCCOLLUM

MOLECULES AND MENTAL HEALTH. Frederic A. Gibbs, Ed. Philadelphia, J. B. Lippincott Co., 1959. 189 pp. $4.75.

For those interested in reading of the relationship between brain chemistry and behavior, the age of scientific togetherness has come: the symposium, the conference, the colloquium, the seminar, the round table, the breakfast table, and the taperecorded coffee-klatch. This trend toward informality may well end by being banned in Boston; indeed, the discussions reported here were "for and by shirtsleeve scientists." Their meetings were intended to cover "new information and concepts," but as with all such symposia, informality threatens to cover up salient lines of argument and evidence. The presentation of new findings in this field has generally been recorded in the proceedings of numerous conferences; these, often, have been as haphazard and difficult to follow as the bulletins scattered throughout the professional and Sunday Supplement literature. Since the editor did not permit interruptions of presentations, one at least can here follow a complete "paper," whatever its wanderings, from beginning to end.

The reports of two conferences held in 1958 under the auspices of the Brain Research Foundation are presented: one, on "Amines in relation to brain function," and another on "ACTH treatment of hypsarythmia." The latter will be of interest to neurologists and pediatricians concerned with a tragic and not infrequently occurring syndrome of infantile spasms, disorganized EEG, and progressive mental retardation. The topic is comprehensively defined by Gibbs, Crawford, and Kellaway among others, and the problems of etiology, pathology, diagnosis, and treatment by Sorel, Low, Lichtenstein, Poser, and Garvin. The relationship of stages of maturation to types of dysrhythmia, and of corticosteroids to both is broached. It is not at all unlikely that vigorous and multidisciplinary approaches in the field of infantile neurological disorders contain models for the work of those concerned with finding twisted molecules for twisted thoughts.

The topics discussed by those concerned with amines and mental disorder are hauntingly familiar: ceruloplasmin, taraxein, plasma oxidases, epinephrine, adrenolutin, LSD-25, atropine—like psychotomimetics, tryptophan metabolism, and central cholinergic stimulants.

The reader will gain insight into the competence and intent of the reported research. From the comments of a number of superbly informed discussants (and the inclusion of bibliographies), he will find a useful critique of these topics. Hoffer, Heath, and Abood present the case for elevated plasma oxidase activity in schizophrenia while the evidence for the metabolic pathways for epinephrine, an assessment of any role of such *in vitro* metabolites as adrenolutin, and discussion of serotonin metabolism is provided by a number of others including Elmadjian, Zeller, Axelrod, Kopin, Kety, and Hoagland. Problems of methodology and of evaluation of psychotoxic effects of serum globulins (such as taraxein is claimed to be) are briefly considered.

No one acquainted with the importance of those topics to neuropharmacology will find this a dull or useless book, for the editor, unlike some of the participants, did achieve a useful covering in depth of the cardinal subjects of current interest through astute programming. Both scholar and neophyte would profit if the scientists with rolled shirt sleeves would labor towards lucid scientific summary of evidence and hypotheses. In the meantime, this work requires less parsing than other symposia of identical content. Given the complexity of the area, the book represents a source wherein one can find the crux of some important current approaches.

DANIEL X. FREEDMAN

PEDIATRIC NEUROLOGY. By Stanley Lamm. New York, Landsberger Press, 1959. 495 pp. $12.90.

This concise textbook was evidently conceived in the author's mind to be a short review of the various neurological diseases which may pass through the doors of a pediatrician's office. Along these lines Dr. Lamm has succeeded admirably, for his book is replete with up-to-date material and each disease is followed by a selected bibliography for additional reference. This book has careful descriptions of such recently described entities as maple syrup disease, Hartnup disease, ataxiatelangiectasia, and metachromatic leukoencephalopathy, just to name a few. In this regard the book is slanted toward the neurologically oriented pediatrician, and care has also been taken to include all pediatric diseases with neurological concomitants or sequelae. For the neurologist, however, this volume is incomplete, for neurophysiological and neuropathological correlations are often too simplified and incomplete.

Though the format of the book aims at conciseness, at times the writing style becomes ponderous and slightly repetitious; brevity of style occasionally seems to be achieved at a sacrifice of preciseness. There is inconsistency in the alternate noting of drug dosage in the metric and apothecary system. This could have been avoided by listing both simultaneously in each instance or by utilizing the metric system, which is to be preferred.

At the beginning of the book there is a short chapter on growth and development, and normative landmarks are listed in tabular form. It would have seemed beneficial, even in a concise book such as this, to include some information on developmental neurology. The work of Peiper, Madame Ste. Anne Dargassies, and Andre Thomas would have been a fitting introduction to the problem of neurological disease in infants and children. Without an awareness of neonatal behavior, both reflexive and volitional, pediatricians will be hard pressed to evaluate a neurological problem in an infant or very young child since so often a slight motor handicap may be the only early suggestion of disease in the first few months of life.

JOSEPH P. ROSSI

RECENT PROGRESS IN HORMONE RESEARCH. The Proceedings of the Laurentian Hormone Conference, 1958, Vol. 15, Gregory Pincus, Ed. New York, Academic Press, Inc., 1959, 495 pp. $12.50.

The annual appearance of The Proceedings of the Laurentian Hormone Conference has come to be an event of importance to endocrinologists throughout the world. The most recent volume lives up to the excellent record of its predecessors. It consists of a total of 14 papers varying from about 15 to 50 pages in length, followed by a transcript of the free discussion which followed the presentation of the papers at the meeting. The papers are concerned with the physiology of simian and human growth hormone, the purification of human gonadotropins, the effects of light on the regulation of gonadotropic activity in birds, steroid protein conjugates and the conjugates of steroids with glucuronic acid and sulphates, the intermediary metabolism of hydrocortisone, factors influencing the secretion of aldosterone, psychological influences on adrenal cortical secretion, the effects of steroids on *in vitro* lymphoid metabolism, and the mechanism of action of parathyroid hormone and of modifications of calcium metabolism. In each of these areas the book presents either a complete résumé of the latest information available on the subject or a novel interpretation of previously available material. For instance, it seems unlikely that anyone will attempt to discuss the physiology of human growth hormone for the next few years at least without referring to the papers by Knobil and Greep, and by Raben. Similarly, Schneider and Lewbart's paper on the fractionation and isolation of steroid conjugates is the most definitive work in the field and contains a good deal of material not yet published elsewhere. Similarly, the studies by Farrell and Bartter and his group on the control of aldosterone secretion represents the frontier of our knowledge in respect to this new and difficult area of physiology.

The book is well written and edited and is a pleasure to read; but in addition it will prove to be a valuable source of information in regard to the subjects it covers for years to come. It is an important link in the continuing chain of knowledge being forged by the Laurentian Hormone Conference. It can be highly recommended to anyone interested in the physiology or biochemistry of the hormones.

PHILIP K. BONDY

THE MOLECULAR BASIS OF EVOLUTION. By Christian B. Anfinsen. New York, John Wiley and Sons, 1959. 228 pp. $7.00.

Proteins are the most important functional and structural components of living systems. Since they are the very basis of phenotype it might be expected that the spectrum of proteins would vary among the great diversity of organisms that have been and are being produced by the evolutionary process. Juxtaposed to this idea might be the conservatism associated with maintenance of function for given protein types, i.e., most random mutational changes would result in enzymes unable to catalyze their specific reactions. With the advent of many new biochemical techniques it is now possible to examine proteins, enzymes, and protein-producing systems in enough detail that critical answers may be obtained to the questions an evolutionarily oriented scientist can ask.

It has been known for some time that parts of certain enzyme molecules can be removed without affecting enzymic activity. Sequence analysis and, more recently, partial analysis of secondary and tertiary structure in relation to biological activity are elucidating rather precisely exactly what parts of the protein are related to the active catalysis. The protein chemist assumes that the remaining area of the molecule are of secondary importance, and therefore more easily varied by evolutionary change without producing profound enzymic effects. Study, then, of similarly functioning molecules of different organism types might turn up difference in these secondary areas of proteins (and in some cases it has). Unfortunately this viewpoint, which is prevalent among some biologists and many chemists, the author among them, ignores the structural role played by enzymes and proteins. Thus these molecular areas that apparently lack functional specificity in the test tube may be of prime importance when the same molecule is in its normal, intricately organized cellular environment.

Topics and discussions of even greater interest than these fill the latter sections of this volume. They serve to summarize the great majority of advances in protein chemistry to the beginning of 1959 and are of great value to the nonprotein specialist in giving him perspective in this rapidly advancing field; even more they will serve to stimulate practising geneticists and evolutionists to think in new terms about the mechanisms of the processes they are studying. Anfinsen is to be lauded for placing emphasis on the cistron in discussions of genetic material—the ambiguity of what a "gene" is can best be overcome by such action.

Introductory discussions of evolution, and classical and modern genetics begin the volume. These are well-written chapters, oriented toward elucidating points the author wishes to emphasize in latter parts of his book; too, they are sufficiently brief that the biologist will not be bored, while the peripatetic chemist will be frustrated into looking further into these subjects.

In summary then, the volume is heartily recommended—rare indeed is the scientist who will not be stimulated by it. The one major objection to the text is the aforementioned lack of consideration of proteins as structural components of cells. Of lesser import are several minor errors that mar the book, a typical case being Anfinsen ignoring the "sense-nonsense"

requirements of DNA base triplets in Figure 80. The book is well bound, beautifully illustrated, and is worth all of its relatively high price.

N.K.W.

AN INTRODUCTION TO BACTERIAL PHYSIOLOGY. By Evelyn L. Oginsky and Wayne W. Umbreit. San Francisco, W. H. Freeman and Company, 1959. xii, 443 pp. $7.50.

This elementary volume of bacterial physiology should serve as a stimulating introduction to the subject. The text reads smoothly and is written in such a manner as to excite the imagination of the uninitiated reader.

Coverage of the subject matter is comprehensive except for the absence of a section on feedback control mechanisms. For the most part the text has been brought up to date, and enough documentation is included to lend force to the discussion, but not so much that the reading becomes tedious. The chapter on bacterial genetics in particular is well written, adequate coverage being given to the topics of transformation, transduction, and recombination. In contrast, however, the chapter on adaptation is an unblended mixture of recent results and concepts, with older outmoded ideas and terminology. There are numerous misstatements scattered throughout the chapter which seem for the most part to be due to careless wording.

The authors have a tendency to present certain hypotheses as being well established explanations for various phenomena. A flagrant example is the section concerning the mechanism of formation and action of diphtheria toxin. Recent work has pointed up the tenuous nature of the evidence supporting the presently accepted hypotheses.

It is felt by the reviewer that, notwithstanding the criticisms mentioned above, this text is a worthwhile addition to the collection of the interested reader.

NORMAN STRAUSS

MIRAGE OF HEALTH. UTOPIAS, PROGRESS, AND BIOLOGICAL CHANGE. By René Dubos. New York, Harper & Brothers, 1959. 236 pp. $4.00.

The author of this book writes more as a philosopher than a microbiologist, yet he reflects on life with the approach of one who has effectively utilized the scientific method in his acquisition of knowledge. *Mirage of Health* contains some of his reflections on life, man, and nature. This distinguished scientist is concerned with the relativity of health and disease and the "ecological equilibrium between microbe and man." He discusses and evaluates numerous factors in history which have had a role in the struggle of man against disease.

Dr. Dubos attaches much importance to the roles of the host and the environment in the etiology and pathogenesis of disease. He draws on numerous examples to support his hypothesis that in many cases disease resides more with the host than with the individual pathogenic organisms. He raises interesting questions in the field of infectious disease. John Snow checked an epidemic of cholera in London in 1854 by removing the handle of the Broad Street pump and thus preventing the use of the con-

taminated water; however, he is quick to point out that experimental subjects have been fed cholera vibrio organisms without developing disease. There are many factors needed, other than the causative organism, to effect an epidemic.

Dr. Dubos casts his lot with the forces of Hygeia, preventive medicine, rather than those of Asclepius, curative medicine, as those which have the greater achievement and offer the greater potential.

This book can be considered pessimistic in some respects; however, Dr. Dubos conceives of man's quest for health as a never-ending stimulus to adventure. *Mirage of Health* is an interesting and exciting book. The author's style is forceful and erudite; throughout, one can perceive the scientist and the philosopher.

WILLIAM L. KISSICK

THE LIFE OF SIR ALEXANDER FLEMING, DISCOVERER OF PENICILLIN. By André Maurois. New York, E. P. Dutton & Co., 1959. 286 pp., 30 illus. $5.00.

Lady Amalia Fleming wrote to André Maurois in November 1955 and requested that he write the biography of her late husband, Sir Alexander Fleming. Perhaps, had the biographer been more familiar with Dr. Fleming during the latter's lifetime, the book might have provided the reader with a more intimate picture of this quiet and taciturn Scot. André Maurois is an author of unquestioned skill and capacity; however, the narrative seems to betray a certain lack of enthusiasm of biographer for his subject. Admittedly, Dr. Fleming was inscrutable during his lifetime and it is no doubt even more difficult to familiarize one's self with him through his papers.

This biography traces Dr. Fleming from his childhood in Ayrshire, Scotland through his medical student days at St. Mary's, his career in the laboratory of Sir Almroth Wright, his excellent work on the treatment of wounds during World War I, experiences with the "magic bullet" Salvarsan, the discovery of lysozyme, the recognition of the inhibitory properties of a penicillium mould on a culture of staphylococci, to the Nobel Prize in Medicine, honorary degrees, and many other recognitions.

Unfortunately, the greater story, which is that of penicillin, is distorted when it is presented primarily as an episode in the life of Fleming. He made the initial observation, but these observations which he reported in *The British Journal of Experimental Pathology* in 1929 contain but a part of the whole picture. Fleming had abandoned penicillin in 1932 as too "labile" for extraction or for therapeutic use. The roles played by Florey, Chain, Heatley, and others in the extraction, purification, and production of the "miracle drug" are not adequately presented.

The prerequisites for biography or autobiography are nebulous. Although the recipient of many honors and the author of much scientific work, Sir Alexander Fleming does not appear to be an ideal subject for a biography.

WILLIAM L. KISSICK

THE LIFE AND LETTERS OF CHARLES DARWIN. Francis Darwin, Ed., New York, Basic Books, 1959. 2 vols., 1,142 pp. $10.00.

The centennial annniversary of the publication of *The Origin of Species* is being marked with a rash of newly published biographies and commentaries on Charles Darwin. The renewed acclaim is not undeserved, for with the perspective that one hundred years lends, his magnificent synthetic exposition is seen to rank with Newton's *Principia* as one of the greatest single published products of Western Science. Today, with the acceptance of organic evolution by most literate men, and with the great changes evolutionary theory has wrought indirectly in philosophy, theology, economics, and the social sciences, it is appropriate to reflect about the mind and character of the Victorian scholar who has so influenced us all.

There is no better place to learn about the man Darwin than from his own pen; fortunately, it is once again possible to do so, for Basic Books has republished Francis Darwin's 1888 collection of his father's letters and autobiography. No modern study can approach this collection in revealing the intricacies of Darwin's life and mind. One reads, for instance, in a last letter before embarking on *H. M. S. Beagle,* the now ironic but then innocent, ". . . I look forward even to seasickness with something like satisfaction." Little did the novice naturalist suspect that four long years of seasickness lay ahead, and indeed, sickness of such severity as to weaken permanently his whole system.

Scientific matters are the subject of most letters in the book. One learns that it was A. R. Wallace who suggested the use of Spenser's term "survival of the fittest," to clarify the meaning of "natural selection." One feels Darwin's sincere surprise and delight on first learning of the Batesian theory of Mimicry. One glimpses the contemporaries—Morgan, Lyell, Wallace, Weisman—and senses the mutual respect and lively intellectual exchange that existed in their circle. But most of all one finds in these letters, which were never intended for public perusal, evidence of the great intellectual honesty, the perseverance, and the imagination which were the prime requisites for elucidating the concept of evolution.

There are, of course, many letters included with only peripheral interest for the general reader. Their presence is not onerous, however, and is no doubt of value to Darwinian scholars. These volumes are clearly the place to satisfy one's curiosity about Charles Darwin.

N.K.W.

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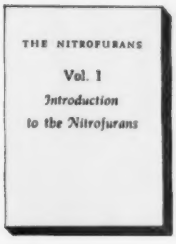

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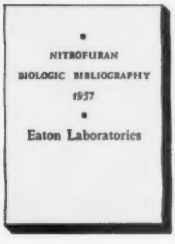

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Edited by Frederic A. Gibbs, M.D.

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